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MYELINATION AND ACETYLCHOLINESTERASE ACTIVITY IN THE DEVELOPMENT OF THE EIGHTH NERVE

ROBERT GUERARD DILLARD

1968

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MYELINATION AND ACETYLCHOLINESTERASE ACTIVITY IN THE DEVELOPMENT OF THE EIGHTH NERVE

By

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A Thesis Submitted to the Faculty
Of the Yale University School of Medicine
In Partial Fulfillment of the
Requirements for the Degree
Of Doctor of Medicine

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1968

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DEDICATION

No parents could have given more in the process of an education. To my mother and father I dedicate this thesis.

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INTRODUCTION

The physiological aspects of the development of hearing in mice have been well studied (Mikaelian & Ruben, 1965, Alford & Ruben, 1963). The morphology of the maturing organ of Corti in that animal has been equally well established following Kikuchi and Hilding's electron microscopic study (1965). As a result of these two publications, several questions have arisen regarding the anatomical correlates of the development of hearing in the mouse.

Mikaelian and Ruben (1965) have shown that nerve action potentials are first recorded at the tenth day of life in mice and that function becomes more sensitive during the next four days. Kikuchi and Hilding (1965) have pointed out that the organ of Corti is essentially mature at the nine day stage. An exception is that the typical efferent endings upon the hair cells are not apparent. Such endings are visualized at the tenth day. Those authors speculate that, possibly, the temporal coincidence of identification of efferent endings and onset of nervous activity is, indeed, more than coincidence.

The complete process of development of the efferent innervation is not established in Kikuchi and Hilding's study. Furthermore, no other electron microscopic developmental studies of that system in the mouse have been attempted. Rossi (1961) has made light microscopic and histochemical observations regarding the development of the efferent innervation in the guinea pig.

The development of myelin may be another factor influencing the onset of hearing. In this paper, the term myelin shall be used to denote multiple layers of fused Schwann cell unit membranes about

nerve fibers or layers of fused statellite cell unit membranes about neuron cell bodies. Myelination shall be the term describing the process of layering and fusing. No studies concerning that process in the eighth nerve of mice are available. Indeed, the mechanism of myelination in the eighth nerve has not been well established in any animal. Rosenbluth (1962) was the first to report the unique ultrastructural features of the myelin sheath of the bipolar ganglion cells in the adult rat and has presented some interesting questions regarding the developmental aspects of the sheath. Eighth nerve cell bodies are usually myelinated, unlike almost all other kinds of neurons. Little or nothing is known about the process of myelination involving nerve fibers in the peripheral eighth nerve.

In this paper, I attempted to study the development of the efferent innervation in the mouse from the level of its origin in the brainstem to the organ of Corti. Using the light microscope, a histochemical technique staining for acetylcholinesterase was employed. Such a technique was used because the efferent but not the afferent component of the eighth nerve contains that enzyme (Churchill, et al., 1956).

A light and electron microscopic study was performed to elucidate the details of myelination in the peripheral eighth nerve. Particular attention was paid to temporal aspects of that process. Morecver, an attempt was made to explain some of the findings of Rosenbluth as well as to correlate anatomical and physiological aspects of the development of the organ of hearing in the mouse.

Literature Concerning The Efferent Cochlear Bundle

The efferent cochlear bundle arises in the contralateral and homolateral superior olivary nucleus in the rostral portion of the medulla, joins the afferent segment of the eighth nerve near the nuclei of that nerve, and courses out of the medulla mixed with the afferent fibers. The efferent component, after sending off branches to the vestibular apparatus, courses in a spiral fashion in the lateral portion of Rosenthal's canal, sending off radial components which ultimately end on the hair cells of the organ of Corti.

Rossi has presented an excellent review of the early literature regarding the spiral fibers (1961). According to him, the first mention of fibers having a spiral course in the cochlea was made by Koelliker in 1859. Boettcher, however, was the first to establish the neural nature of the spiral bundle (1859), considering the fibers to be extentions of the cells of the spiral ganglion.

It was Held (1909) who pointed out that the spiral fibers course from the modiolus to the organ of Corti without interruption in the spiral ganglion. Borghesan (1930) strengthened Held's hypothesis by experimental studies in which he severed the acoustic nerve at its point of exit from the medulla and observed that the spiral fibers degenerated before damage to the cells of the spiral ganglion was noted. The degeneration was similar to that seen when peripheral axons were severed.

Studying the origins of the efferent bundle, Papez, in 1930, showed that the fibers of the superior olivary nucleus became a part of the

contralateral eighth nerve. In 1942, Rasmussen, using degenerative techniques, succeeded in following the course of the olivary fibers to the basal turn of the cochlea in guinea pigs. Technical difficulties prevented further tracing at that time, but eleven years later, Rasmussen (1953) was able to show that the fibers from the superior olive on the contralateral side passed into the spiral bundle earlier described by Koelliker, Boettcher and Held.

Fernandez (1951) and Portmann et al. (1953) combined their work with the work of Held and Rassmusen and deduced that fibers from the contralateral superior olivary nucleus coursed in the spiral bundle and terminated on the inner hair cells of the organ of Corti. However, no direct anatomical evidence was available to confirm such a deduction. Rasmussen in his 1953 paper stated: "However plausible and interesting the current conception may be that the efferent fibers terminate on the internal hair cell receptors on the cochlea, it would appear that further confirmation by reliable investigative methods is necessary before considering this important point definitely settled."

Such evidence began to appear in 1956 with the publication of Churchill, Schuknecht, and Doran's work entitled "Acetylcholinesterase Acitivity in the Cochlea." Using a histochemical technique involving the deposition of cuprous thiocholine with subsequent treatment to form CuS (Koelle et al., 1949), sites of acetylcholinesterase activity were localized to the region of the inner and outer hair cells and to certain nerve fibers oriented toward the organ of Corti in the osseous spiral lamina. It was pointed out by the authors that previous studies

of nerves elsewhere in the body showed negligible amounts of acetylcholinesterase in afferent sensory components. Thus, they made the interesting proposal that perhaps what had been observed by them had been the efferent fibers described by Rassmusen.

The definitive work appeared in 1959 (Schuknecht et al.). It was shown that by cutting the fibers of the superior olivary nucleus as they crossed the midline in the brainstem, the staining reaction for acetylcholinesterase at the level of the organ of Corti was markedly diminished, proving the connection between the superior olive and the hair cells.

The next step to take place in the study of the efferent bundle and its course from the brainstem occurred in 1960 when Rassmusen described the homolateral component of the olivocochlear bundle. This bundle originated from the superior olivary nucleus on the same side, joined its crossed component lateral to the genu of the facial nerve, then proceeded to the organ of Corti. Rossi (1965) observed still a third set of fibers originating in the reticular formation of the pons and medulla passing in an ipsilateral fashion into the exiting eighth nerve.

In 1954, Engström and Sjöstrand published the first paper on the ultrastructure of the hair cells of the organ of Corti and their nerve endings. At that time, they pointed out that, in general, the nerve endings on the inner hair cells were larger and more heavily granulated than were endings located about the outer hair cells. A more thorough study was performed by Engström in 1958. In it, he was able to show quite definitely that two different types of nerve endings existed on the hair cells of the organ of Corti. One

species was generally small, sparcely granulated, and measured from 0.5 to 1.0 micron in diameter. The second type was significantly larger, measuring from 1.0 to 3.0 micra and filled with a larger number of vesicles measuring 200 to 500 Å (Engström, 1960). The synaptic membrane of the hair cell apposed to these larger endings was, in all cases, curiously double. These types of endings were present under both inner and outer hair cells, but it appeared to Engström that the distinction between the two was less significant under the outer cells. Engström hypothesized in view of the dual innervation of the organ of Corti that perhaps the heavily granulated endings were of an efferent nature, while the smaller endings were afferent.

Since 1958, several workers (Spoendlin, 1963; Smith, 1961a; 1961b) have confirmed Engström's earlier work. Smith (1961c) has further identified the smaller nerve endings as having "synaptic bars" similar to structures existing in the retinal rod synapses. Thus, further weight was given to the opinion that the smaller endings were afferent (as all retinal rod synapses are afferent).

Hilding and Wersäll presented a paper in 1961 in which they further strengthened the above hypothesis. Working with Holmstedt's modification of Koelle and Friedenwald's thiocholine method for acetylcholinesterase localization (Holmstedt, 1957), they reported precipitates in close relationship to the internal spiral bundle and in the large granulated endings under both inner and outer hair cells. The outer hair cells showed a progressive diminution of staining moving towards the helicotrema. In the apical turns of the cochleas studied, only the nerve endings below the inner most of the outer hair

cells reacted. The smaller, sparcely granulated cells were seen never to react. When taken with the findings of Schuknecht et al. (1959), it was felt that precise localization of the cholinesterase activity under the hair cells at the large endings gave fairly conclusive indirect evidence of their efferent nature.

The first direct evidence came when Iurato (1962) showed that by cutting the contralateral and homolateral components of the eighth nerve in adult rats, one could visualize degeneration of the large granulated (Type II) endings in the cochlea. The small endings were left undisturbed. Kimura and Wersäll (1962) and Spoendlin and Gacek (1963) in similar but more thorough experiments essentially confirmed Iurato's findings.

Function Of The Efferent Bundle

Studies of the function of the efferent bundle were made by Galambos in 1956. He showed that by stimulating the efferent bundle as it crossed midline in the medulla of anesthetized cats, the action potential in response to sound stimulus was diminished as recorded indirectly at the round window. Fex (1959) confirmed Galambos' findings. He showed further that such stimulation of the efferents also increased the size of the cochlear microphonics (CM) at the round window. (It will be remembered that the CM is most probably a reflection of the receptor potential generated at the hair cell level. Davis, 1961) In a later report, Fex (1962) studied individual fibers of the eighth nerve and showed that electric stimulation applied to the efferent nerve (with macroelectrodes)

inhibited the discharge of individual afferent fibers excited by external sound stimuli. The homolateral component of the efferent system has also been shown to be of an inhibitory nature (Desmedt et al., 1963).

The last point to be discussed in reviewing the literature concerning the physiology of the efferent innervation is the rather confusing point regarding inhibition of the efferent inhibitory system. One would expect that the copious amount of acetylcholinesterase activity would point to the hypothesis that acetylcholine might be the chemical mediator at the efferent endings. However, cholinesterase inhibitors have been shown to have no effect on the activity of the efferent bundle. Strychnine, a well known blocker of inhibitory activity in the CNS does abolish the effects of electrically stimulating the efferent fibers (Desmedt et al., 1961).

Developmental Aspects of the Anatomy and Physiology of the Organ of Hearing

The embryogenesis of the eighth nerve and the organ of Corti has been well delineated. Mall (1888) described the eighth nerve ganglion in dog embryos as being the connecting link between the early ear vesicle and the neural tube. The entire mass of the presumed neuroectodermal tissue and vesicle was surrounded by a mesodermal capsule. Streeter (1907) confirmed Hall's descriptions using human specimens. He felt that nerve fibers developmentally arose from the ganglion anlage and in some manner became connected with the neural tube to form the cochlear division of the eighth nerve.

Parenthetically, his theory regarding the development of nerve fibers is interesting, if quite incorrect. He stated "that the cochlear trunk consists originally of a column of ganglion cells connecting the anlage of the spiral ganglion with the brain, and the conversion of this column into fibroblasts produces the early fibers of the trunk". It seems obvious now that his "fibroblasts" were, in reality, nerve fibers growing centrally into the brainstem.

Streeter (1918) has written an excellent review of the early literature concerning the embryology of the inner ear. Apparently, Reissner (1854) and Reichert (1854) were the first to show that the cochlear duct was originally an ectodermal tube, being an outgrowth of the immature vesicle. Retzius (1884) in his now famous monograph formally set down a description of the formation of the organ of Corti, showing that it developed as a thickening in a portion of the ectodermal tube with subsequent cell type differentiation. Since his exquisite studies, the light microscopic picture of the development of the organ of Corti has been essentially unchanged.

The first developmental study concerning the efferent cochlear bundle was made by Rossi (1961). Using the Koelle and Friedenwald technique for acetylcholinesterase localization, he was able to show that a positive reaction in the region of the spiral bundle first occurred in the basal turn of the cochlea at the 90 mm. stage in guinea pig fetuses. At that stage, the cochlea showed completion of the scala vestibuli and scala tympani along its entire length.

The tectorial membrane and spiral ligament were also quite evident throughout. The organ of Corti consisted of rudimentary columnar cells. With progressively more mature fetuses, the spiral bundle was seen to grow in an apical direction. At the 93 mm. stage, a positive reaction for acetylcholinesterase was seen in the basal turn on the level of the rudimentary cells of the organ of Corti. In the latter case also, as the animals were matured, the positive reaction ascended. By birth, all but the apical coil showed such activity. It was not until the forty-third day of life in the guinea pig, however, that cholinesterase activity was noted in the apical turn of the organ or in the apical spiral bundle. At that time, the organ of Corti was completely differentiated. It is noteworthy that the reaction in the spiral bundle consistently preceded temporally the development of cholinesterase activity in the organ of Corti.

Kikuchi and Hilding have studied the development of the organ of Corti in the mouse using the electron microscope (1965). Those workers showed that although typical afferent nerve endings were apparent at birth, no identification of efferent endings could be made until the tenth day of life. At birth, the mouse organ of Corti was quite rudimentary, the majority of the cells in the region being of the columnar type. Future hair cells and supporting cells resembled each other but could be identified, however, in electron micrographs. By ten days, the organ appeared to have an essentially adult form.

Mikaelian and Ruben (1965) have made an electrophysiological study of the development of hearing in a normal strain of mice. No evidence of cochlear microphonics (CM) was seen before the eighth day of life. One-half of all eight day old mice showed CM responses. By the tenth day, all animals tested exhibited the CM as recorded at the round window. By the fourteenth day, the potentials had the adult ranges and sensitivity.

Action potentials as reflected by recording indirectly at the round window began to appear at the ninth day. All ten day animals tested in a similar manner showed the response. Latencies decreased daily until normal adult values were seen at the fourteenth day of life.

History of the Study of Myelin

From its inception up to and including the present time, the literature concerning myelin and myelination has been distinquished by the confusion and disagreement which it displays. An excellent historical review of these topics is contained in an article by Münzer (1939) appearing in "The Quarterly Review of Biology"; pertinent points from this work, based largely upon a study of the original writings, will be summarized.

It appears that the first description of myelinated nerve fibers was given by an Italian physician, Felice Fontana, in 1781. Observations made on teased preparations of nerve fibers resulted in the statement that they were "composed of transparent, homogeneous, uniform, very simple, cylinders". There was at that time and for several decades to come, no distinction made between the nerve itself and its myelin sheath.

The next milestone in the study of this area of neurohistology occurred in 1816 with a publication of Treviranus' On the Organic Elements of the Animal Body. This author pointed out that nerve fibers were "skinny tubes which were filled with a tough matter the myeline (sic.) (nervenmark) proper, and were held together in bundles by sheaths of cellular tissue." This work was the first to contain an accurate drawing of a peripheral nerve with encasing sheath and was also the first to use the term nervenmark or "nerve-marrow" which has since been translated into English as "myelin". Unfortunately, Treviranus used the term to denote the contents of his "tubes" and not the wall itself. Thus, it is obvious that confusion with the study of myelin begins with the name itself, "myelin" being a derivative of the Greek word myelos meaning marrow. (Stedmann's Medical Dictionary, 1966).

The first study to deal with the development of nerve fibers was that of Robert Remak, published in 1836. He noticed that in newborn animals, nerve fibers contained no encasement as had been well described in adult animals and that the formation of myelin advanced at different rates in animals of different species.

In 1839, Thedor Schwann published his monumental. Mikroskopische Untersuchungen über die Uebereinstimmung in der Struktur und dem Wachstum der Thiere und Pflanzen. (Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants). In addition to the idea set forth for the first time that living matter was completely made up of cells, Schwann was the first to describe the myelinated nerve fiber in detail and speculate on its development (Causey, 1960). In order to

understand his description, it is first necessary to explain his theory regarding the development. From Schwann's studies, he was of the opinion that nerve fibers began as "primary nerve cells" which were oriented along the route of the future mature nerve. These primary cells ultimately coalesced, forming a syncitium, or the secondary cell. Following the formation of the syncitium, a deposition of white material (myelin) appeared upon the inner surface of the secondary cell membrane. The adult nerve fibers then consisted of (1) an external membrane "structureless...which appears to be minutely granulated. This membrane presents itself as a narrow, clear border, which is readily distinguished from the dark contours of the white substance". Within this membrane, infrequently appeared nuclei which Schwann believed to be remnants of the primary cell nuclei; (2) the nerve sheath consisting of "white substance"; (3) the central "Band of Remak" (known now to be the axon) (Ibid.). From this description, Schwann considered the sheath and external membrane, later to be given his name, as an integral part of the neuron itself. Essentially, however, he did describe the structure of a peripheral neuron correctly, but he was confused regarding its development and composition.

The discovery separating the axon and sheath of Schwann anatomically apparently took place gradually within the next thirty years after the publication of Schwann's work. In 1878 Ranvier, in his Lecons sur L'histolgie due Systeme Nerveux (Studies on the Histology of the Nervous System) made it clear that the "Band of Remak", as described by Schwann,

was an outgrowth of the nerve cell body and not a part of the "secondary cell". In his study, Ranvier described the proliferation of Schwann cells in nerves distal to sites of injury and gave the first description of the nodal interruptions of the myelin sheath, now known as Ranvier's nodes. He quite clearly pointed out that there was only one nucleus in the sheath between two of these nodes (Ranvier, 1878).

However, Schwann's syncitial theory of nerve formation was not by any means overthrown by Ranvier's work. As late as 1907, Bethe was writing concerning the neurogenic properties of the Schwann cell, especially regarding regeneration of nerve fibers (Bethe, 1907).

It was Ramon y Cajal who was able to solve the argument concerning the relationship of the axon to the Schwann sheath when he published his book on the histology of the nervous system in 1909. Cajal was for the most part in agreement with Ranvier's notions but clarified and strengthened the details considerably. He was able to present a surprisingly accurate drawing which is shown in figure 52 as reproduced from his work on the peripheral myelinated axon. In it can be recognized the separation of the Schwann sheath and axon, Lantermann incisures, nodes of Ranvier, a Schwann cell nucleus, and the sheath of Mauthner, or axolemma (Ramon y Cajal, 1909).

Such a picture of peripheral fibers was generally accepted until the appearance of electron microscopic studies in the early 1950's . However, in the intervening period, several studies were performed regarding the nature of the nodes of Ranvier and internodal distance, especially in relation to neurophysiology (Erlanger and Gasser, 1937;

Hursh, 1939). The chemical nature of myelin was clarified; and in 1939, Schmitt and Bear, studying birefringence in the myelin sheath, came to the conclusion that myelin consisted of "concentric sheets of protein interspersed between layers of lipoids so as to form structures which are repeated periodically in a radial direction". Using low angle X-ray diffraction techniques, the same authors with Clarke and Palmer, were able to obtain measurements of the radial repeating unit and reported it to be on the order of 100 Angstrom units for fresh mammalian tissue (Schmitt, Bear, and Clarke; 1935; and Schmitt, Bear, and Palmer; 1941).

Thus, up to the advent of the use of electron microscopy as applied to studying nervous tissue, it was conjectured by optical birefringence and X-ray diffraction studies that myelin surrounding nerve fibers was arranged in a lamellar fashion.

Electron Microscopy of Myelin in the Peripheral Nervous System

When Sjöstrand (1949) and Fernandez-Moran (1950) published their studies based on electron micrographs of nerve tissue, the first evidence microscopically was obtained of the lamellar structure of myelin. In Sjöstrand's work, fragmented layers of myelin were visible after metallic coating of homogenates of nerve fibers. Fernandez-Moran studied myelinated nerve fibers in situ and described a series of parallel lines with regular spacings of eighty to ninety Å in rat sciatic nerves cut transversely. He was unable to conclude whether such figures completely surrounded the nerve fibers.

Sjöstrand in 1953, published the first clear photograph of a cross section of a myelinated nerve fiber, stating that the lamellated structure was continuous about the individual fiber (1953). His measurements put the spacing at approximately 120 Å between the repeating thick lines.

By 1957, Robertson was able to publish high resolution electron micrographs of the myelin sheath showing clearly its lamellar nature. He described a repeating unit consisting of a major dense line of approximately twenty-five Å and an intraperiod space of 100 Å, with an intraperiod (minor) line centered in the intraperiod space. (See figure 75)

Geren studied the process of the formation of the myelin sheath using the electron microscope (1953, 1954). She discovered that the structure arose as a result of concentric wrapping of the Schwann cell about a nerve fiber. Figure 76 illustrates the process (Ham. 1961). It can be seen from the diagrams that as the process begins, the Schwann cell embraces and encircles the axon (Figure 76,A) and appears quite similar to the adult unmyelinated axon. The Schwann cell then begins to rotate about the nerve (Figure 76,B). In C, it can be seen that the cell membrane lining one side of the groove comes to lie in apposition to its counterpart lining the opposite side. As the process continues, the cytoplasm existing between two unit membranes disappears leaving such membranes in contact with each other. As stated earlier in this paper, the mature form of the myelin sheath is apparent when the cytoplasm has disappeared and the membranes have fused.

It must be noted that in Figure 76, the unit membrane is represented as a single line. In actuality, that structure has three layers as shown in figure 75, i.e., an outer and inner layer of protein separated by a thickness of lipid. The entire membrane measures approximately seventy-five Å.

In elucidating the fine structure of the myelin sheath, Robertson (1957) was able to identify the precursors of the repeating unit. He noted that that unit was composed of a double thickness of cytoplasmic unit membranes. The major dense line formed from fusion of two inner layers of cell membrane; the intraperiod space resulted from two thicknesses of lipid becoming approximated; and the intraperiod line arose from fusion of two outer layers of membrane. The process is well illustrated in figure 75.

Peterson et al., (1955), using tissue cultures of avian spinal ganglion, were able to describe revolutions of the Schwann cell cytoplasm about axons in vitro, offering confirmatory evidence for the above theory. Ernyei et al., (1966) showed by time lapse photography that pure cultures of Schwann cells energetically wrapped themselves about artifical "nerve fibers" in a spiral manner.

However, the simplicity of the "jelly-roll" hypothesis of myelination in the peripheral system has been seriously questioned on several different accounts. Peters and Muir (1959) published a photograph showing a Schwann cell containing one large and several small myelinated axons. In 1963, Dixon studying the fifth nerve ganglion in the rat, was able to point out several different Schwann cells containing multiple myelinated axons. Furthermore, Webster et al., (1960) observed

many myelin irregularities in the sciatic nerve of guinea pigs inexplicable by the spiral process of myelination as presented above. Indeed, those workers estimated that such irregularities occur in 75% of the largest myelin sheaths.

The structure of the unmyelinated axon has been much less puzzling. It is felt that the Schwann cells simply envelop one to several axons and give the appearance of being an early attempt at myelination (Robertson, 1959; Hess, 1959).

During the late 1950's, the ultrastructure of the nodes of Ranvier (Uzman, née Geren, 1957) and Schmitt-Lantermann clefts (Robertson, 1958) was well delineated, so that at present, the anatomy of the peripheral nerve fiber is understood with some degree of confidence.

Electron Microscopy of Myelin in the Central Nervous System

The ultrastructure of myelin and myelination in the central nervous system has been significantly more difficult to describe. The sheath itself is similar in structure to its peripheral counterpart. There are certain exceptions, however. (1) The outer sleeve of cytoplasm beyond the denser lamellations usually contains much less cytoplasm (Maturana, 1960). (2) Similarly, the inner ring of cytoplasm may have much less cytoplasmic material, to the point of being obliterated (Gray, 1964). (3) Peters has pointed out dense radial striations in CNS myelin and concluded that such markings are a result of a material within the minor line (1961). (4) Finally, redundant myelin sheaths without axons, or covering several different axons have been described in the toad cerebellum by Rosenbluth (1966).

The process of myelination in the central nervous system has been confusing because of the multiple variations in both gross and microscopic anatomy observed in the more complex brain and spinal cord. Several different theories regarding the process have been described, the earliest being that of Luse (1956). She suggested that several oligodendroglia abut against a single axon, contributing their membranes to form a myelin sheath after fusion of the concentric layers. Ross et al., (1960) reporting in vitro studies of rat cerebellar tissue cultures showed that during myelin formation, many oligodendroglial cell processes arranged themselves in a complicated multilamellated array. Myelination then occurred by flattening of the individual cell processes and fusion of the cell membrane to form a portion of the lamella. Such a process is quite similar to that proposed by Luse.

DeRobertis et al., in 1958, proposed a mechanism apparently occurring in the spinal cord of rats and cats involving a process by which the central axon bored through the glial cytoplasm. They arrived at such a conclusion because they were unable to locate mesaxons in their studies. They advanced the hypothesis that within the oliogodendroglial cytoplasm, vesicles were formed, which grew, flattened, and fused to form the lamellated sheath.

A third theory derived mainly from studies of the optic nerve in amphibia and mice advanced the proposal that myelination occurred essentially in the same manner exhibited in the peripheral nervous system, i.e., by the "wrapping" hypothesis. (Uzman, et al., 1960; Peters, 1961, 1964). Bunge et al., (1962, 1965) modified and strengthened such an hypothesis

by showing that in neonatal kitten spinal cords, oligodendroglia sent out processes which surround axons separated by a considerable distance from the glial cell body and myelinated them in a spiral fashion. Such a cell was frequently seen to be myelinating several axons. Similar processes in the peripheral nervous system have not been described.

Rosenbluth (1966), by studying the toad cerebellum, described redundant myelin sheaths and offered still another mechanism by which myelination could take place in the central nervous system. He postulated that the process occurred in two phases. First, a simple "spiral" was formed about an axon or cell body, and secondarily, redundant sheaths developed as a result of active overgrowth of the myelin at paranodal edges or at the Schmitt-Lantermann clefts. Such overgrowths could then engulf or partially engulf other axons or cell bodies in the adjacent tissue.

In mammals, myelinated cell bodies outside the CNS occur only in the vestibular and spiral ganglia of the eighth nerve. The anatomy of these two structures was first studied in detail with the electron microscope by Rosenbluth and Palay (1961), using the eighth nerve ganglia of the goldfish. Their work revealed that three general types of ensheathments occurred about the neuron cell body. First, there was the infrequent occurrence of a simple Schwann cell encapsulating a neuron. Such a picture is quite similar to that observed in the dorsal root ganglion of the rat (Rosenbluth and Palay, 1960). Second, and most commonly, the encapsulation of neurons by a sheath consisting of "loose" myelin was seen. The loose sheath consisted of from two to many lamellae

arranged so that the majority of the lamellae contain cytoplasm. Frequently, one part of a ganglion cell was seen to be encased by loose myelin while an adjacent segment of the same cell showed dense myelin of the type described below. No discontinuities were seen in the lamellae. The third type of encapsulation occurred about the larger neurons and consisted of compact myelin similar in structure and periodicity to that seen in peripheral myelinated axons. Additional findings included the observation that the number of lamellae covering the perikaryon (synonymous with neuron) was several times less than the number enveloping the corresponding axons and that the neuron cell body may have more than one satellite cell surrounding it.

One year later, Rosenbluth published a study of the cochlear ganglion of the rat (1962). Certain important differences were observed when the same structures in the rat and goldfish were compared. First, the number of lamellae of the "compact" type of sheath surrounding the rat eighth nerve ganglion cells is significantly less. Second, although with phase contrast studies, there appeared to be loose and compact myelin, it was seen with the electron microscope that, in fact, there was much less subdivision into the two specific categories in the rat. There was, moreover, no evidence of cell bodies encapsulated by "simple" satellite cells. Most significantly, however, was the observation that rat perikaryal (neuronal) sheaths exhibited multiple lamellar discontinuities and irregularities. Several types were mentioned: (1) lamellae ending as blind loops of cytoplasm in the middle of the sheath; (2) the number of lamellae in any given capsule was inconstant; (3) some lamellae were seen to change direction in their course about

the cell body and double back; (4) internal and external mesaxons at times faced in the same direction instead of the opposite as would be expected if a spiral process were implicated; (5) several sheaths showed more than one internal or external mesaxon; (6) and finally, adjacent lamellae were seen to fuse to form a single lamella. From these findings in the adult rat, Rosenbluth deduced that in some cases, perikaryal myelin developed in a pattern different from the traditional mechanism proposed by Geren. He postulated several mechanisms by which myelination could occur.

The first was that of "interlocking spirals" in which the two Schwann cells would send out cytoplasmic processes over the neuron, laying down successive pairs of lamellae. The advancing edges in this case would have to insinuate themselves between the perikaryon and the lamella already deposited. An alternate process with similar results could take place if the two satellite cells rotated together in toto about the neuron or if satellite cells remained fixed, and the neuron rotated. The second proposed mechanism involved "superimposed spirals" in which one spiral would be laid down on top of another completed spiral.

Both of these processes failed to explain all of the irregularities seen. The third postualted mechanism involved "overlapping folds" in which case, several lamellae would fold back upon themselves. Again, however, Rosenbluth felt that the observed structures were much more complicated than that simple hypothesis could explain.

The last process mentioned was one in which the development of perikaryal myelin could occur in a totally haphazard manner. One or more satellite cells would send out sheaths of cytoplasm in all directions over the neuronal surface, crossing and overlapping each other at random. A significant point made by Rosenbluth in his discussion was that compact perikaryal myelin showed no such irregularities.

Materials and Methods

Cholinesterase Study:

Animals were sacrificed at birth, 3, 5, 7, 9, 11, 22 days and maturity (usually 100 days). A total of twenty normal albino and pigmented mice were studied. After anesthetizing each animal with ether, the thorax was opened, the inferior vena cava severed, and from 15 to 100cc of unbuffered 10% formalin (depending upon the age of the animal) was injected into the left ventricle or the ascending aorta. The animal was then decapitated; the head was skinned and cleaned of its muscle and periosteum following which one of two different techniques was used. For sections of the whole head, the mandible was removed with adjoining soft tissue, and the forepart of the skull was separated at approximately eye level. The remainder of the head was placed in cold NaOH buffered (pH 7.4) 10% EDTA in a refrigerator at 4°C. (Balogh et al., 1964). The solution was stirred with a magnetic stirrer. Young animals generally decalcified within twenty-four hours. Adults required forty-eight hours.

In a second group of animals, the head was submerged in 10% formalin and dissected using a Zeiss operating microscope. The cranium was opened, and the brain carefully dissected out. The temporal bones were then separated and the bullae removed. The remaining cochleas were placed in 10% EDTA and treated in a manner similar to that used in the treatment for whole heads.

The material to be sectioned (brain, whole head, cochleas) was rinsed briefly with distilled water and frozen on a Jung CO_2 freezing block. Specimens were transferred to a Jung-Dittes cryostat at

18°F. and sectioned at twenty micra. Tissue slices were transferred from the knife to a lightly albuminized coverslip using a fine brush. The coverslips were removed from the cryostat and allowed to dry at room temperature for thirty minutes. Using a method described by Gomori (Pearse, 1961), the coverslips were next placed in an incubating medium for cholinesterase determination. The stock solution contained:

$CuSO_4$	0.30 gm.
Glycine	0.375 gm.
$MgCl_2$	1.00 gm.
Maleic acid	1.75 gm.
NaOH (4%)	30.00 cc.
$NaSO_4$ (40% hot saturated)	170.00 cc.

To 15 cc. of the stock solution were added thirty mg. of acetyl thiocholine iodide to prepare the incubating medium.

The tissues were incubated at 37°C. for one hour then rinsed in three changes of saturated $Na_2\ SO_4$. Next, they were immersed for two minutes in 1% $(NH_4)_2S$ and then finally rinsed in distilled H_2O and mounted on slides with glycerine jelly. Specimens were examined using a Zeiss photomicroscope.

Inhibition of Cholinesterase:

Newborn and adult specimens were prepared in a manner similar to that described above except that before incubating them in the acetyl thiocholine-Gomori medium, they were preincubated in a 10^{-5}

concentration of the inhibitor BW c251 for thirty minutes. They were then placed in the Gomori medium to which had been added the inhibitor Bw c251 to make a total concentration of 10^{-5}, incubated, and treated similarly to the specimens prepared simply for cholinesterase activity.

Light Microscopy Using a Phospholipid Stain for Myelin:

Animals were sacrificed at birth, 3,5,7,9 days, and maturity (approximately 100 days). A total of eight animals were studied. After anesthetizing each animal with ether, the thorax was opened, and the animal was perfused in a manner similar to that described above but using 4% cacodylate buffered (pH 7.4) glutaraldehyde solution. After decapitation, the heads were skinned and cleaned. The temporal bones were removed or the whole head was utilized. After removing the tympanic bullae of the temporal bones, the cochleas or the whole heads were placed in 10% EDTA and treated as above for twenty-four to forty-eight hours. The decalcified specimens were rinsed in distilled water, frozen, and sectioned at twenty micra in the cryostat, using the procedures outlined above. Tissue slices were stained for phospholipids (lecithins, cephalins, and sphingomyelins) using a modification of Baker's method (Baker, 1946). Coverslips were (1) placed in a solution containing 5.0 gm. of K_2Cr_2O7, and 1.3 gm. of $CaCl_2$ and 100 cc. distilled water at 60°C. for one hour; (2) next, they were rinsed in water; and (3) placed for three hours at 60°C. in a solution containing 0.1 gm. hematoxylin and 0.02 gm. $NaIO_3$ and 100 cc. of water. (after that solution had been stirred for one hour) (4) the tissues were washed for five minutes in distilled water and

(6) then differentiated for approximately eighteen to twenty-four hours in a solution containing 0.25 gm. $K_3Fe(CN)_6$, (7) finally, they were washed in distilled water and mounted on slides with glycerine jelly. Phospholipids stained a blue to dark blue.

Electron Microscopy:

Animals were sacrificed at birth, 3, 5, 7, 11, 22 and 100 days. Nine animals were studied. The mice were treated similarly to those used in the light study with an emphasis on speed. After perfusion with 4% buffered glutaraldehyde and decapitation, the temporal bones were removed, and the bullae and outer wall of the cochlea were dissected away leaving the modiolus and attached organ of Corti intact. All dissections were performed in cold glutaraldehyde using the operating microscope. The dissected specimens were left in glutaraldehyde at 4°C. for approximately one hour and then placed in cold cacodylate buffer overnight. They were then treated with osmic acid for one hour and dehydrated. Next they were placed in gelatine capsules filled with Epon plastic and allowed to harden at 60°C. for forty-eight hours. A fine saw mounted on a watchmaker's lathe was used to prepare the specimens for sectioning. Freehand sections of the organ of Corti, spiral ganglion and modiolar eighth nerve were made for phase contrast microscopy. An LKB Ultratome was used for preparing sections for study with an RCA EMU 3G electron microscope. Contrast was improved by staining with a saturated solution of uranyl acetate in 50% ethanol.

Results

Development of the Efferent Bundle and the Organ of Corti

Newborn Mice

Brainstem:

In the newborn mouse, the entire efferent bundle is apparent in the brainstem as defined by its cholinesterase activity (Figure 1). It can be followed in serial sections to the point of its exit with the eighth nerve. As opposed to the adult stage, there is very little other cholinesterase activity apparent, making identification of the bundle relatively easy.

Spiral Ganglion and Peripheral Eighth Nerve:

Significantly, the entire spiral ganglion and peripheral eighth nerve within the modiolus stains for cholinesterase. Rossi (1961) observed activity in the region to become the spiral ganglion in guinea pigs at the 34 mm. embryonic stage. At such a point in development, the cochlea was only begining to grow into its spiral form; the organ of Corti was not recognizable even in an immature form. By the 85 mm. stage, such activity had disappeared. At that age of maturity, the cochlea was moderately well differentiated, the scala vestibuli and scala tympani begin present throughout. The immature organ of Corti consisted of columnar cells and appeared to be similar in structure to that in the newborn mouse. At no point in the guinea pig's development did cholinesterase activity appear in the eighth nerve. Whether the marked activity noted in the present experiment is located in the nerve fibers or their sheath cell cytoplasm cannot be determined.

With the phase contrast microscope, typical bipolar neurons are apparent in the spiral ganglion surrounded by a thin sheet of satellite cytoplasm (Figure 2). Rarely, two satellite cell (to be used synonymously with Schwann cell) nuclei are seen about the same ganglion cell.

Figure 3 is a photograph of the lateral portion of the spiral ganglion of a newborn mouse in the region where the efferent spiral bundle is located in the adult mouse. Arrows point to structures which may represent that spiral bundle.

Organ of Corti:

Anatomical findings as related to the organ of Corti in normal mice at birth are similar to those reported by Kikuchi and Hilding (1965) and by Mikaelian and Ruben (1965). With the phase contrast microscope (Figure 4), it is observed that the hair cells are low columnar with large basally located nuclei having several dense nucleoli and a thick membrane in osmium fixed tissue. The inner cells appear to be taller than the outer. Hairs are apparent at the top of both inner and outer cells. Pillar cells are present as is a large spiral vessel. There are no fluid spaces apparent. The tectorial membrane extends to the hair cells, is flat, and immature. The area between the limbus and inner hair cells and under the tectorial membrane is filled with tall pseudostratified cells with basally located nuclei and clear cytoplasm. The apices of these cells abut against the inferior margin of the tectorial membrane. Nerve fibers can be seen passing through the habenula perforata (Figure 5).

Cholinesterase activity is present in the region of the hair cells at this stage although the reaction is moderately feeble (Figure 53). Intense staining is seen in the cells lateral the hair cells up to the stria vascularis. The region of the osseous spiral lamina shows moderate activity.

Three Day Mice

Brainstem:

At this stage, little change can be seen in the brainstem. The efferent bundle is clearly evident.

Spiral Ganglion and Peripheral Eighth Nerve:

The eighth nerve continues to stain in toto for cholinesterase activity from its point of egress to its termination on the organ of Corti (Figure 55). The spiral ganglion is similar in appearance to that of the newborn stage when studied by the phase contrast microscope. Unmedullated fibers can be observed in the region of the spiral bundle, most probably representing efferent fibers (Figure 6).

Osseous Spiral Lamina and Organ of Corti:

Here also, there is little change in structure or cholinesterase activity (Figure 7). There are no spaces of Nüel, and the pillar cells continue to be opposed. There is no space between the tectorial membrane and the pseudostratified columnar cells. Fine nerve fibers can be seen passing through the habenula. Cholinesterase activity is present in the region of the nerve fibers of the osseous lamina and the immature hair cells.

Five Day Mice

Brainstem:

There is no change from the three day stage. The brainstem continues to be relatively immature in so far as cholinesterase activity is concerned.

Spiral Ganglion and Peripheral Eighth Nerve:

The peripheral eighth nerve continues to show cholinesterase activity in its entirety (Figure 56).

Osseous Spiral Lamina and Organ of Corti:

Cholinesterase activity is apparent in the lamina and at the base of the hair cells. The activity in the latter region is somewhat more intense (Figure 57).

The organ of Corti continues in its immature form (Figure 8). A space is beginning to appear beneath the tectorial membrane at its limbic connection as the columnar cells begin to shrink. No fluid spaces are apparent. Mikaelian and Ruben (1965) found that the tunnel of Corti is opened at the fourth day in their strain of mice whereas Kikuchi and Hilding's mice (1965) did not develop a tunnel space until the sixth day at the basal turn, and it was not completely open until the tenth day. In the present study, it is apparent that at the fifth day, the tunnel is not yet open.

Seven Day Mice

Brainstem:

There is no change from previous descriptions of the efferent system. In general, however, considerably more cholinesterase activity is seen.

Peripheral Eighth Nerve and Spiral Ganglion:

The only cholinesterase activity present at this stage appears in the efferent bundle (Figure 58). The major component of the eighth nerve and the cells of the spiral ganglion no longer show activity. The activity of the efferent bundle stands out strongly against the absence of activity elsewhere. Its fibers can be seen coursing individually and in small groups into the spiral bundle, and radial fibers can be seen passing out to the organ of Corti (Figure 59).

Organ of Corti:

The organ of Corti shows considerable change when compared with its five day counterpart (Figure 9). This space beneath the tectorial membrane has enlarged significantly as the columnar cells continue to shrink. The tunnel of Corti is open to the lower part of the apical turn. Spaces are apparent between the outer pillar and the outer hair cells. The spiral vessel is less prominent. Cholinesterase activity is present in the hair cell region.

Nine Day Mice

Brainstem:

There is no change from the seven day stage.

Peripheral Eighth Nerve and Spiral Ganglion:

No cholinesterase activity in the major component of the eighth nerve nor in the spiral ganglion cells is seen. The efferent bundle is vividly apparent and similar in appearance to its counterpart in the seven day stage.

Osseous Spiral Lamina and Organ of Corti:

The osseous lamina shows definite evidence of myelinated fibers and strong cholinesterase activity in the efferent fibers (Figure 10).

The organ of Corti with phase contrast studies at this stage appears to be nearing maturity (Figure 11). The formerly psuedostratified columnar cells situated beneath the tectorial membrane are now shrunken to a cuboidal configuration. The tunnel of Corti is fully open, and fluid spaces are apparent between the outer hair cells as well as between the inner most outer hair cell and outer pillar. The spiral vessel is now less prominent than at any preceding stage. Cholinesterase activity is limited to the region underneath the hair cells (Figure 10).

Eleven and Twenty-two Day Mice

There are only subtle changes in either morphology or cholinesterase activity in the eleven or twenty-two day mice when studied with the light microscope and compared with the nine day stage. The fluid spaces in the organ of Corti grow larger and the hair cells are becoming completely separate (Figure 12).

Adult Normal Mice

Brainstem:

The contralateral efferent bundle can be followed from its origin in the superior olivary nucleus to its point of egress from the brainstem. Because of the myriad of cholinesterase positive fibers present in the adult, the homolateral component cannot be visualized with certainty (Figures 60,61).

Peripheral Eighth Nerve and Spiral Ganglion:

The efferent component is easily recognized as it travels with the afferent fibers to its termination on the hair cells (Figure 13). Typical bipolar ganglion cells, seemingly encapsulated, are seen with phase contrast studies.

Osseous Spiral Lamina and Organ of Corti:

As in animals of ages 7, 9, 11 and 22 days, the efferent bundle is clearly apparent in the osseous spiral lamina. Terminations upon the hair cells are distinct (Figure 62).

The organ of Corti has changed little from its nine day stage (Figure 14). The fluid spaces are larger and the outer hair cells are completely separate.

Inhibition of Acetylcholinesterase Activity with BW c251

In the newborn and adult mice treated with the specific acetylcholinesterase inhibitor, BW c251, the following results are obtained:

Newborn

No staining is seen in the pathway of the eighth nerve from its entrance into the modiolus to the hair cells. Slight staining continues to be present in the cells of Claudius between the outer hair cells and the stria vascularis (Figure 63). For comparison, figure 64 shows a similar section of a newborn mouse cochlea which is stained for cholinesterase without inhibition of specific acetylcholinesterase. It is observed that the eighth nerve stains in toto as does the area of the hair cells of the organ of Corti, the cells of Claudius, and the stria vascularis.

Adult

The spiral ganglion, efferent spiral bundle, osseous lamina, and organ of Corti presented in figure 65 show no evidence of staining. For comparison, figure 62 shows a similar section from an adult mouse which is stained for cholinesterase without inhibition of specific acetylcholinesterase. Activity is seen in the osseous lamina and in the region beneath the hair cells.

From the above inhibition studies, the lack of enzyme activity in the regions in question allows the conclusion that the cholinesterase activity previously observed in both the newborn and adult mice in uninhibited specimens is, in fact, that of specific acetylcholinesterase.

Myelination in the Spiral Ganglion

Newborn Mice

Light Microscopy:

Light studies of the peripheral eighth nerve using a stain specific for phospholipids reveal no evidence of myelin, either of the nerve fibers or neurons (Figures 66, 67). Phase contrast microscopy confirms the lack of myelin sheaths (Figure 15). When the spiral ganglion is visualized, typical bipolar neurons are seen (Figure 2). Each such cell body is surrounded by one or rarely two satellite cells containing a dark crescentic shaped nucleus and thin cytoplasm which appears to envelop the entire cell. Unmyelinated nerve fibers in the region of the spiral bundle are seen (Figure 3).

Electron Microscopy:

Neuron Cell Bodies:

The neurons are seen to be large and generally round on thin section (Figure 18). The poles of these cells are rarely visualized concomitantly. Rosenbluth (1962) likewise commented on the rarity of seeing the typical bipolar processes on thin section and noted that such a situation resulted from the poles of the cells not being directly opposite each other.

The cytoplasm contains a generous amount of rough endoplasmic reticulum (ER), multiple dense cylindrical mitochondria scattered throughout, and Golgi apparatus (Figure 17). Multivesicular bodies described by Rosenbluth are occasionally seen, but "heterogeneous" bodies (1962) are not apparent in the tissue studied. No subsurface

cisterns, also noted by Rosenbluth, are present at this stage. All cell bodies studied in the newborn animal are of similar composition and most like the "granular" type described by Rosenbluth. The nucleus is large, finely granular and contains one eccentric nucleous (Figure 18).

Satellite Cells:

In all instances, only a single layer of satellite cell cytoplasm is present over the surface of the neuron (Figures 16-19). In several cells viewed, the cytoplasm is discontinuous, the two leaves abutting each other (Figures 17-19). Rarely, the two leaves overlap each other for short distances (Figure 20). Many processes can be seen branching from the satellite cell cytoplasm. A typical "T" formation is seen in figure 18. In most instances, the processes are more like that seen in figure 24, i.e., a single branching offshoot.

The cytoplasm of the satellite cell is relatively dense (Figures 16-19). The perinculear region is filled with copious rough ER and infrequent Golgi apparatus (Figure 16). The mitochondria are generally round with only rare oblong figures present. The coarse intramitochondrial granules noted by Rosenbluth in adult rat satellite cell cytoplasm, and seen in renal (Palade, 1952) and intestinal epithelial tissue (Weiss, 1955), are scarce in the sheath cell cytoplasm at the one day stage. Most mitochondria appear in regions located on either side of the nucleus, but it is common to see such figures in the peripheral cytoplasm at the opposite poles as well (Figure 19). The ER is generally

limited to the perinuclear region and is similar in appearance to neuronal ER (Figure 16). Clear vacuoles with dense walls are occasionally present in the peripheral cytoplasm (Figure 18). The satellite cell cytoplasm is bounded by a regular unit membrane which is, in turn, covered by a basement membrane (Figure 17). Interestingly, in figure 17, a satellite cell can be seen engulfing an adjacent nerve fiber simultaneously.

Nerve Fibers and Schwann Cells:

Nerve fibers are the same as those described elswhere (Rhodin, 1963). Neurotubules, neurofilaments, and mitochondria are present (Figure 21). The mitochondria are distinguished at this stage by their characteristic central cristae which bissect the mitochondria sectioned transversely. In the same figure, a Schwann cell can be seen engulfing a nerve fiber in a manner strikingly similar in appearance to the figures published by Geren (1954) in her study on the development of myelination. Adjacent to that structure lie a great number of fibers incompletely surrounded by multiple Schwann cell processes. Many of the isolated processes have "captured" more than one axon. On the right in the same photograph, a cytoplasmic process from the main cell body of a Schwann cell can be seen to wrap three such axons. A similar situation was mentioned in the spinal cord of kittens by Bunge et al., (1962,1965) in which oligodendrioglia sent out long processes and myelinated axons at a considerable distance from the cell body.

The Schwann cell nuclei appear similar to those in the perikaryal satellite cell (Figure 21).

The cytoplasm is also similar to satellite cell cytoplasm with the following exceptions: (1) Golgi apparatus are more frequent and are perinuclear (2) Endoplasmic reticulum of a different nature is frequently seen (Figure 21), the granules being considerably larger, and the distances separating the two laminae greater. The distinction between the Schwann cell which envelops nerve fibers and the satellite cell which envelops neurons or perikaryal cells may be purely artifical. As noted in the observations above, in some cases the satellite cell has been seen to wrap nerve fibers simultaneously. However, to avoid confusion, in this paper, Schwann cells shall be defined strictly as those cell enveloping nerve fibers and satellite cells as those enveloping neurons.

Three Day Mice

Light Microscopy:

Light studies reveal no significant change from the one day stage.

Electron Microscopy:

Neuron Cell Bodies:

There is no change in the neuronal configuration.

Satellite Cells:

Figure 22 shows a tongue of isolated satellite cell cytoplasm inserted between the perinuclear cytoplasm of the satellite cell and the neuron.

In the adjacent neuronal complex, a tongue of cytoplasm can be seen to "sprout" from the internal surface of the satellite cell and insert itself between that cell and the neuron. A pseudopod-like process can be seen branching from the satellite cell at (P). Large vacuoles bounded by very thin membranes are present in the perinuclear region of both cells. Such structures are not seen in the one day specimens. Otherwise, the three day satellite cell cytoplasm is identical to that in the newborn. In figure 23, another instance of a connection existing between the perikaryal sheath cytoplasm and the cytoplasm investing a nerve fiber is seen. Figure 24 shows a single branching offshoot of the satellite cell cytoplasm.

Nerve Fibers and Schwann Cells:

Figure 25 shows nerve fibers at a slightly more advanced stage of envelopment then appears in the one day specimens studied. There is otherwise no change noted in the configuration of the nerve fibers or Schwann cells.

Five Day Mice

Light Microscopy:

The sections stained for phospholipid as seen in figure 68 have a blue-green color outlining the nerve fibers as they course to the spiral ganglion seen on the right hand side of the photograph. A higher magnification (Figure 69) gives a better picture of the process. The oblong, tan-colored nuclei are those of Schwann cells. The ganglion cells are also faintly outlined as seen in figure 70. A high magnification phase contrast photomicrograph (Figure 26) shows a single densely outlined

segment of a nerve fiber. Another longer segment is seen in the osseous spiral lamina (Figure 27). In the specimens prepared in this manner, myelination is not nearly so prevalent as in the phospholipid stained specimens. A temporal discrepancy may exist. The configuration of the spiral ganglion as studied by phase microscopy at the five day level shows no change. In figure 28, a typical ganglion cell is seen to be ensheathed by two satellite cells.

Electron Microscopy:

Neuron Cell Bodies:

Several large clear vacuoles (mb) are apparent in the cytoplasm of the cell on the right in figure 29. They appear to have a single, dense, thin membrane and are similar in structure to the multivesicular bodies described by Rosenbluth (1962) in the adult rat bipolar ganglion cells. Such figures are also seen in the one day animals (Figure 17). The adjacent cell in figure 29 shows four large vacuoles filled with finely granular material. Such figures are not seen in younger animals. There is otherwise no change in the neuronal configuration.

Satellite Cells:

Little advance is made in the configuration of the perikaryal sheath (i.e., the satellite cell) structure. Figure 31 shows a typical "clubbed" process of a satellite cell. Large, irregular, clear vacuoles are present. Similar vacuoles are seen in figure 12 of Rosenbluth's (1962) paper. Such structures are seen also in one day mice.

Nerve Fibers and Schwann Cells:

No myelinated figures are present in the five day specimen studied with the electron microscope. Most nerve fibers observed appear to be encircled by from one to two layers of Schwann cell sheaths and are similar in appearance to figures observed in younger mice. Figure 30 shows such a fiber cut slightly obliquely. In figure 32 is seen an axon hillock region in which the nerve fiber portion is ensheathed by two layers of Schwann cell cytoplasm as denoted by the arrows. From the photograph, it is not possible to ascertain whether the perikaryal and perineural cytoplasm is continuous.

Seven Day Mice

Light Microscopy:

Tissue stained for phospholipid shows slightly more intense staining in the region of the nerve fibers than occurs in the five day specimens. Resolution in the tissue studied is not so good as that obtained in the five day specimens because of poor fixation. However, the blue coloration is more widespread (Figure 71), extending higher into the apical region. With phase contrast microscopy, nerve fibers are seen to be encased in thin, dark sheaths signifying accumulation of lipids i.e. the formation of myelin. All fibers seen show such a configuration. Fibers in the region of the spiral ganglion cut longitudinally as in figure 33 show up well. Moreoever, fibers within the osseous spiral lamina at the apex are likewise so ensheathed (Figure 34). No change can be seen in the spiral ganglion neurons or their satellite cells.

Electron Microscopy:

Neuron Cell Bodies:

For the first time, a neuron of the type described by Rosenbluth as "filamented" is seen (Figure 36). Its mitochondria are more sparse, and fine filaments are present throughout the cytoplasm. Otherwise, the cytoplasmic organelles are similar to those seen in the "granular" type cell which, in all the sections studied up to this point, has been the only cell type seen. At (S) in figure 36 is a structure described by Rosenbluth (1962) as a "subsurface cistern". He interpreted such structures as being modifications of the endoplasmic reticulum. They are typically flattened against the neuronal plasmalemma and separated from it by a zone of less than 130 Å.

Satellite Cells:

Still no marked change can be seen in the structure of the perikaryal sheaths (i.e., the satellite cells). In many places, the sheath consists of double layers of satellite cell cytoplasm, but the majority of the cells studied show only a single layer. At (AH) in figure 35, the region of an axon hillock is shown. The junction between the perikaryal cell and the perineural cell is shown by an arrow. There is no apparent continuity between the cells. The perineural cytoplasm is significantly thicker.

Nerve Fibers and Schwann Cells:

Nerve fibers in transverse section appear unchanged with the exception that the "single crista" mitochondria have been replaced by "multiple cristae" type as seen in figure 36 at (M).

The most striking change is seen in the Schwann cell configuration. For the first time with the electron microscope, light myelinated axons are seen (Figure 36, 37). Many relatively unadvanced stages of myelination can be seen in the seven day specimens. It is puzzling that intermediate stages are not observed.

Nine Day Mice

Light Microscopy:

Tissue stained for phospholipid shows marked coloration about the nerve fibers (Figure 72). Significantly, only slight staining is noted in isolated regions of the spiral ganglion. The regions of the spiral bundle and osseous lamina stain strongly. With phase contrast microscopy, nerve fibers are noted to be somewhat more heavily myelinated when compared with the seven day stage (Figure 38). No change can be detected in the bipolar ganglion cells or their perikaryal sheaths (Figure 39).

Eleven Day Mice

Light Microscopy:

Phase microscopy reveals a little change from the nine day stage. In rare neuronal complexes, the perikaryal sheath appears slightly more dense (Figure 40). Myelinated axons are prevalent.

Electron Microscopy:

Neuron Cell Bodies:

No change can be seen in the eleven day neurons when compared with the seven day cells.

Satellite Cells:

Figure 42 shows an area of overlap of the perikaryal cell cytoplasm with a vacuole (v) present in the clubbed shaped termination. Multiple psuedopodia are seen in the same sheath. Four layers of cytoplasm can be seen between the parallel lines in figure 41. In general, at least two layers are present around the bipolar ganglion cells at this stage.

Nerve Fibers and Schwann Cells:

Nerve fibers show no change. The width of the myelin sheaths has increased considerably from the seven day stage, and myelinated figures predominate (Figure 41). Stages of very early envelopment continue to be present although intermediate stages are not seen.

Twenty-two Day Mice

Light Microscopy:

Perikaryal sheaths are considerably more dense at twenty-two days. Each neuronal cell body is so encompassed (Figure 43).

Electron Microscopy:

Neuron Cell Body:

No change can be observed when the twenty-two day specimen is compared to its earlier counterparts.

Satellite Cells:

A striking increase is observed in the number of satellite cell lamellations and in their density. At this stage, ten to fifteen layers exist. Figure 44 nicely demonstrates the changes observed.

Nerve Fibers and Schwann Cells:

Nerve fibers themselves show no change from their earlier configuration. The process of myelination of nerve fibers has advanced as denoted by increased thickening of the sheath. Significantly, in figure 45, multiple cytoplasmic bridges can be seen among the sheaths of the nerve fibers. Several such connections are denoted by arrows in that figure. At (C) in that photograph, a connection exists between the cytoplasm of a satellite cell and the cytoplasm of a Schwann cell.

Adult Mice

Light Microscopy:

Tissue stained for phospholipid evidences intense coloration in the nerve fiber region (Figure 73). In addition, a band of blue outlining each cell in the spiral ganglion is histochemically indicative of perikaryal myelination. Phase microscopy reveals dense myelin sheaths about nerve fibers. Each ganglion cell is seen to have a myelin sheath (Figure 46) which is approximately two times as wide as its twenty-two day counterpart.

Electron Microscopy:

Neuron Cell Body:

No change is observed in the bipolar ganglion cell cytoplasm (Figure 47). Because of poor fixation, mitochondria are seen in varying stages of disruption.

Satellite Cells:

From ten to twenty lamellae are present. Figure 48 shows a high magnification of a perikaryal sheath with nineteen layers. The outer layer contains much cytoplasm and is usually discontinuous in several places over the surface of the sheath. Another "T" formation can be seen in figure 49. The multiple irregularities noted by Rosenbluth within the sheath itself are not seen with frequency, although resolution in the present study due totechnical shortcomings is not so good as his.

Nerve Fibers and Schwann Cells:

Nerve fibers have not changed from their earlier configuration with the exception alluded to earlier regarding the appearance of the cristae of the mitochondria. In the studies of myelin sheaths, several interesting findings are noted. Cytoplasmic bridges as in the twenty-two day stage are common between sheaths of different nerve fibers. Figure 50 shows possible connections among four nerve fiber sheaths. Other areas suggestive of connections are seen in figure 51. Unmyelinated axons, though quite rare, are also seen in the same figure. A node of Ranvier is apparent in figure 49.

Review of Findings

Table one summarizes in the first five columns results obtained in the present series of studies. Results relating to developmental aspects of anatomy and physiology obtained by other workers appear in the subsequent columns. Briefly, in the newborn and three day mice, it has been shown that there is no evidence of myelin (i.e., multiple layers of fused Schwann cell or satellite cell unit membranes) at any point in the peripheral eighth nerve. Nerve fibers are generally partially or totally ensheathed by one layer of Schwann cell cytoplasm. A single layer of cytoplasm surrounds the bipolar ganglion cells.

At the same stages, acetylcholinesterase activity is present about the peripheral portion of all components of the nerve. Unfortunately, the generalized cholinesterase activity appearing in young mice precludes identification of the efferent component in the peripheral nerve by its differential staining characteristics. Phase contrast studies of one and three day mice inconclusively point to the presence of the efferent bundle in those stages at the level of the spiral bundle. The efferent system in the brainstem is, however well developed in the newborn mouse.

At the five day stage, fairly widespread myelination of nerve fibers in the lower turn of the cochlea appears as evidenced by faint positive staining for phospholipids in that area. Phase microscopy reveals a rare dense sheath signifying myelination in the same region.

The discrepancy in the extent of the process at this stage has two possible explanations. First, the presence of phospholipids in the Schwann cell cytoplasm may precede demonstrable lamellation of the sheath; or secondly, there may be a temporal difference in age of specimens examined. No significant change can be noted in the appearance of the perikaryal sheaths. The organ of Corti shows beginning shrinkage of the pseudostratified columnar cells beneath the tectorial membrane. The tunnel of Corti is not open.

By seven days, nerve fibers with several layers of compact myelin are common although many unmyelinated figures persist. There has been relatively little change in the perikaryal sheath configuration, but a marked change has appeared in cholinesterase activity. Only the efferent component of the eighth nerve shows such activity. The afferent fibers and the spiral ganglion cells are non-reactive. The efferent system can be followed to the region of the hair cells. The space beneath the tectorial membrane has enlarged considerably and the tunnel of Corti is open at least to the low apical turn. Fluid spaces are present.

By nine and eleven days, myelination of the nerve fibers is generalized throughout. Nine day specimens stained for phospholipid show marked coloration in the region of nerve fibers even in the apical turn. At eleven days, there has been some progression of lamellation in the perikaryal sheaths but no compact myelin is present.

Cholinesterase activity remains limited to the efferent system. The organ of Corti shows further maturation at these stages. The tectorial membrane has reached its adult configuration by nine dyas, and the tunnel of Corti is fully open. Space of Nüel are of the mature size.

At twenty-two days, myelination of axons appears similar to that in the adult picture. Perikaryal sheaths aregenerally of the compact lamellated structure. Cholinesterase activity is limited to the efferent system. Only the contralateral component of the olivo-cochlear bundle can be identified with certainty in specimens treated for cholinesterase activity.

Discussion

Anatomic, Enzymatic, and Physiologic Development of the Inner Ear

Development of the Efferent Bundle:

The presence of the complete contralateral component of the olivo-cochlear bundle in the brainstem at birth, its possible presence in Rosenthal's canal at the same time, and its definite presence in the canal at the seven day stage is significant. Kikuchi and Hilding (1965) in their study of the development of the organ of Corti in mice point out that recognizable efferent innervation is not present at the hair cell level until the tenth day of life. Two possibilities exist. The efferent endings may be present at the hair cell level but so immature as to be indistinguishable from the afferent endings; or the peripheral portion of the nerve may continue

to be approaching the hair cells at a point between the spiral bundle and the hair cells, i.e., in the osseous spiral lamina. From the results obtained in the present study, no conclusions can be made.

Early Cholinesterase Activity:

Another point of interest is the extensive acetylcholinesterase activity present in the entire peripheral eighth nerve during the first few days of life. Rossi (1961) reported a slightly positive reaction for cholinesterase in the region of the future spiral ganglion neurons in the guinea pig beginning at the 25 mm. stage and ending at the 71 mm. stage. During that period, the anatomy of the organ of Corti is considerably less mature than that of the newborn mouse. Generalized activity is not seen in the fibers of the eighth nerve at any stage in the guinea pig. In adult cats (Gacek et al.,1965), the afferent fibers show no cholinesterase activity, but neurons in the spiral ganglion do show such activity. Thus it appears that there is species variation, both developmentally and in the adult animal.

Kupfer et al., (1957) have measured the total cholinesterase activity quantitatively in homogenized rat forelimbs at different ages. They were able to show that maximum concentrations of cholinesterase appeared shortly after birth and declined thereafter. Such results correlate well with the histological evidence of accumulation of cholinesterase in the entire peripheral eighth nerve of young mice.

The physiological significance of such intense activity is puzzling in the young mice studied. It cannot be determined from the present set of results whether the acetylcholinesterase activity is localized in the nerve fibers themselves or in their Schwann cell cytoplasm. Nonspecific cholinesterase, but not acetylcholinesterase, activity has been noted by Gerebtzoff (1964) in the myelin sheaths of peripheral nerve fibers with light microscopy. This activity was most intense about the nodes of Ranvier. In an electron microscopic study of nonspecific cholinesterase and acetylcholinesterase activity in adult cholinergic nerve fibers (Lewis and Shute, 1966), it has been shown with exquisite detail that no nonspecific or acetylcholinesterase activity is present in any part of the myelin sheath. In view of the meticulous precautions taken against artifactual diffusion, the latter study seems most likely correct.

No deductions regarding precise localication of cholinesterase in the present study of immature animals can be made with certainty, but in view of the above findings, it appears likely, at any rate, that the enzyme activity is localized to the nerve fibers. Histochemical electron microscopic studies are necessary before conclusions can be made safely.

Lack of acetylcholinesterase activity in adult mice is fairly good indirect evidence that acetylcholine is not involved in neural transmission in the afferent system. The corallary to that statement, i.e., that the presence of acetylcholinesterase activity in the

afferent system of young animals means that acetylcholine is involved in early transmission is not necessarily true. First, the afferent system is not transmitting impulses at that stage as evidenced by Mikaelian and Ruben's work (1965). Second, there is indirect evidence that some nerve fibers containing acetylcholinesterase do not utilize acetylcholine as their neurohumoral transmitter substance. Specifically, the efferent eighth nerve component contains large amounts of acetylcholinesterase, but its action on nerve potentials is not inhibited by classical cholinesterase inhibitors (Desmedt et al., 1961). The above factors lead one to suspect some other function for the acetylcholinesterase activity present in the eighth nerve of young mice.

Function of Early Cholinesterase:

It is interesting that, temporally, the process of myelination of nerve fibers is beginning to take place just prior to the time that acetylcholinesterase activity is waning, i.e., at the five day stage. By the seven day stage, myelinated fibers predominate, and at that time, acetylcholinesterase activity in the entire afferent component has disappeared. Speculation concerning anything but a temporal relationship is just that. However, it may be possible that in some as yet undiscovered fashion, acetylcholinesterase activity may influence the process of myelination in nerve fibers. One must remember, however, that while the enzyme activity is lost in the cells of the spiral ganglion at the same time, the process of myelination of those structures does not take place until much later.

Development of the Organ of Corti:

The developmental pattern of the mammalian organ of Corti has been well known since studies by Retzius (1884) on the maturation of that structure in the rabbit. Similar studies of the mouse organ of Corti have been most recently performed by Kikuchi and Hilding (1965) and Mikaelian and Ruben (1965). Observations obtained in this study correspond with the temporal sequence of events persented by the above workers. In mice, the organ of Corti changes from a fairly undifferentiated ectodermal tube at birth to its adult form by approximately the tenth day.

Anatomical and Physiological Correlates:

When the results obtained from research performed by this observer are added to the findings of Kikuchi and Hilding (1965) and Mikaelian and Ruben (1965), a rather complete picture of the anatomical and physiological development of the organ of hearing in mice is obtained. As regards present findings, by the seventh day, myelinated nerve fibers are commonplace, but at that age, no electrical activity in the form of cochlear microphonics and nerve action potentials is obtainable. It is not until twenty-two days that bipolar ganglion cells are myelinated, yet, as noted, adult physiologic values are present at fourteen days. Thus it appears that there is no correlation between myelination and onset of function, except that myelination of nerve fibers precedes electrical activity by at least two and probably four days. However, it is seen from Table 1. that latencies of the N_1 component of the activity as recorded at the round window decrease steadily as the process of myelination in general

becomes more widespread and as the thickness of the individual sheaths is increasing. Although as seen from the table, many other features of the organ of hearing are developing at the same time, myelination probably plays some role in the approach of physiologic values to their adult level.

Acetylcholinesterase activity is present in an essentially adult configuration at the seven day stage. Therefore, it appears that such activity is not one of the crucial factors related to the onset of hearing in mice as measured by several parameters, but it is to be assumed that it is a necessary prerequisite.

It will be noted that in Mikaelian and Ruben's studies that one-half of the animals tested at eight days showed the cochlear microphonic phenomenon although the range of stimulus frequencies eliciting a response was small. By nine days, N_1 responses were present in those animals who exhibited CM's responding in a stimulus frequency range between 600 and 8,000 cycles per second. It was only at the tenth day that eighth nerve action potentials as recorded in the nerve itself appeared. The temporal discrepancy of one day between the N_1 and R_1 (Signifying eighth nerve action potential recorded directly) is discussed by Mikaelian and Ruben (1965) and Ruben et.al., (1962). They conclude that N_1 may be a measure of activity in the nerve fibers peripheral to the spiral ganglion and that R_1 represents activity of nerve fibers more centrally located. The delay of one day may represent a differential rate of maturation. No significant anatomical difference in the present study has been

noted between the seven and eleven day cells of the spiral ganglion. Furthermore, using the light microscope, there appears to be little difference in the degree of myelination at the nine day stage when comparing fibers in the osseous lamina and ones central to the spiral ganglion. Electron microscopic studies concerning this point have not been done.

Results of the present study indicate that onset of hearing develops after myelination of nerve fibers and the appearance of an adult configuration of acetylcholinesterase. Indeed, all of the contents of the cochlea with the exception of the perikaryal sheath are essentially mature before physiologic values are obtained.

However, Hilding et al., (1967) have reported that hearing responses in normal mink are present while the organ of Corti in that animal remains immature. Such a result is confusing and difficult to explain on the basis of present concepts regarding the mechanism of auditory function. Further studies into this matter are called for before valid assumptions can be made.

The Process of Myelination

Myelination of Nerve Fibers:

Several interesting findings have been noted in studying the process of myelination. Figures of the type described by Geren (1954) in which a Schwann cell envelopes a nerve fiber in the now classic pre-"jelly roll" configuration are common in the newborn and three day specimens. Three, five, and seven day animals exhibit slightly more

advanced wrapping. However, intermediate stages containing more than four or five layers of cytoplasm in a "loose" spiral situation have not been observed in the present study. A sampling error is unlikely; rather, it is conjectured that once initiated, the process of wrapping and dusion occurs rapidly, making observations of intermediate structures statistically improbable. Many myelinated axons of the "peripheral" type are seen in older animals, internal and external mesaxons being clearly visible and a simple spiral configuration apparent.

A type of "atypical" myelination also has been shown to be present among the specimens studied. In the newborn animal, groups of nerve fibers are seen to be in the process of being enveloped by cytoplasmic processes from Schwann cells located at some distance from the fibers themselves. Similar structures are present in three day specimens. Again, no intermediate stages are observed, but interestingly, in the adults examined, possible cytoplasmic bridges between myelinated fibers are fairly common, offering indirect evidence that the early figures have gone on to myelinate the fibers they were surrounding. Such a process has not been observed in the peripheral nervous system up to the present time. Bunge et al., (1962) have reported that a similar process exists in the spinal cord of kittens. In their study, oligodendroglia were seen to have cytoplasmic bridges connecting the main body of the cell and nearby axons.

In the process of myelination, such findings mean that the entire Schwann cell body does not rotate about the nerve fiber. However, the spirals are seen to be regular in their configuration so that either the

the fiber rotates on its axis or the cytoplasmic process of the Schwann cell progressively grows inward. In view of studies regarding the process of myelination about inanimate axons in vitro (Ernyei, 1966), it does not appear likely that the nerve fiber itself rotates.

Typical unmyelinated fibers have been observed in the adult stages. Rosenbluth (1962) observed similar structures in his study. The function of such fibers is not known.

Myelination of Neuron Cell Bodies:

Little change can be seen in the configuration of the perikaryal sheath until the eleven day stage. Before that time, however, several interesting findings related to the process of myelination of the cell bodies are seen. At the one day stage, perikaryal sheaths consist of one layer of cytoplasm. It is often seen to be discontinuous at one point, the two layers abutting against each other or one layer insinuating itself beneath the other for a short distance. Rosenbluth pointed out a similar situation in adult goldfish but noted that such figures do not exist in adult rats. In the present studies, no neurons are seen to be enveloped by two satellite cells when studied with the electron microscope. Rarely, such a situation is seen with phase contrast studies. The most striking and characteristic finding involving the immature sheaths is their ability for forming pseudopodial processes, both on the inner and outer aspects of their cytoplasm. At times, even "T" formations of such offshoots can be observed, i.e., a small offshoot sending its branches off in opposite directions.

From these observations and others such as isolated "tongues" of cytoplasm seen between layers of regular perikaryal cell cytoplasm, it is apparent that at least the initial process of myelination is a quite haphazard procedure.

At the eleven day stage, as many as four layers of cytoplasm have been observed about a cell body. Generally, however, at that stage, only two such layers are seen. By the twenty-two day period, ten to fifteen layers of dense myelin are common.

In the adult, the perikaryal sheaths observed were all of the "compact" type described by Rosenbluth. No "simple" sheaths were noted. Perhaps as a result of technical inexperience, irregularities as observed by him in the adult rat perikaryal sheaths were not seen.

However, an adequate basis for all of the irregularities so described by Rosenbluth can be found in the hypothesis that the offshoots observed in young mice in the present study, both on the inner and outer aspects of the sheath cytoplasm continue growing about the cell in a spiral fashion, fusing in places to form the typical compact sheath, and doubling back on themselves in places accounting for mesaxons oriented in the same direction as noted by Rosenbluth.

Such a process must take place relatively slowly with the majority of activity occurring between the eleventh and twenty-second day of life in the mouse.

Additional Findings

Findings not directly related to the study of the process of myelination have also been reported here. In several photographs, possible connections existing between the cytoplasm of perikaryal sheaths and perineural sheaths raise the question of whether a single Schwann cell can ensheath a nerve fiber and a neuron concomitantly. Such findings render distinctions between Schwann cells and satellite cells questionable and unnecessary.

The two types of ganglion cell neurons identified by Rosenbluth (1962) are apparent in the present study. From the numbers observed, however, it seems that the "granular" type far out number the "filamentous" type.

Summary

1. The literature concerning the study of the efferent component of the eighth nerve is reviewed.

2. A general review of the literature of the study of myelination is presented beginning with the first descriptions of nerve fibers in the eighteenth century and including recent electron microscopic findings. Particular attention is paid to Rosenbluth's studies regarding the aantomy of myelination in the acoustic ganglion.

3. A developmental study of the process of myelination in the mouse spiral ganglion is presented using both light and electron microscopy. It is shown that myelination of nerve fibers begins at the fifth day of life and exists in its adult configuration by the twenty-second day. Two essentially different methods of myelination are seen. Perikaryal myelination begins shortly after birth, but the process is quite slow in comparison to that involving the nerve fibers. The major part of perikaryal myelin is produced between the eleventh and twenty-second day. It is herein proposed that the adult configuration is produced by spiral growth of the satellite cell about the neuronal cell body with multiple branching of the cytoplasm.

4. The acetylcholinesterase activity of the central and peripheral eight nerve is studied. It is shown that the efferent bundle as identified by its cholinesterase activity is present in the brainstem

in its entirety at birth. Further, it is seen that the entire peripheral eighth nerve stains positively for acetylcholinesterase activity until the fifth day after birth. After that time, only the efferent bundle is cholinesterase positive.

5. Finally, the relationship between myelination and the development of cholinesterase activity to physiological aspects of development of hearing in mice is discussed.

APPENDIX

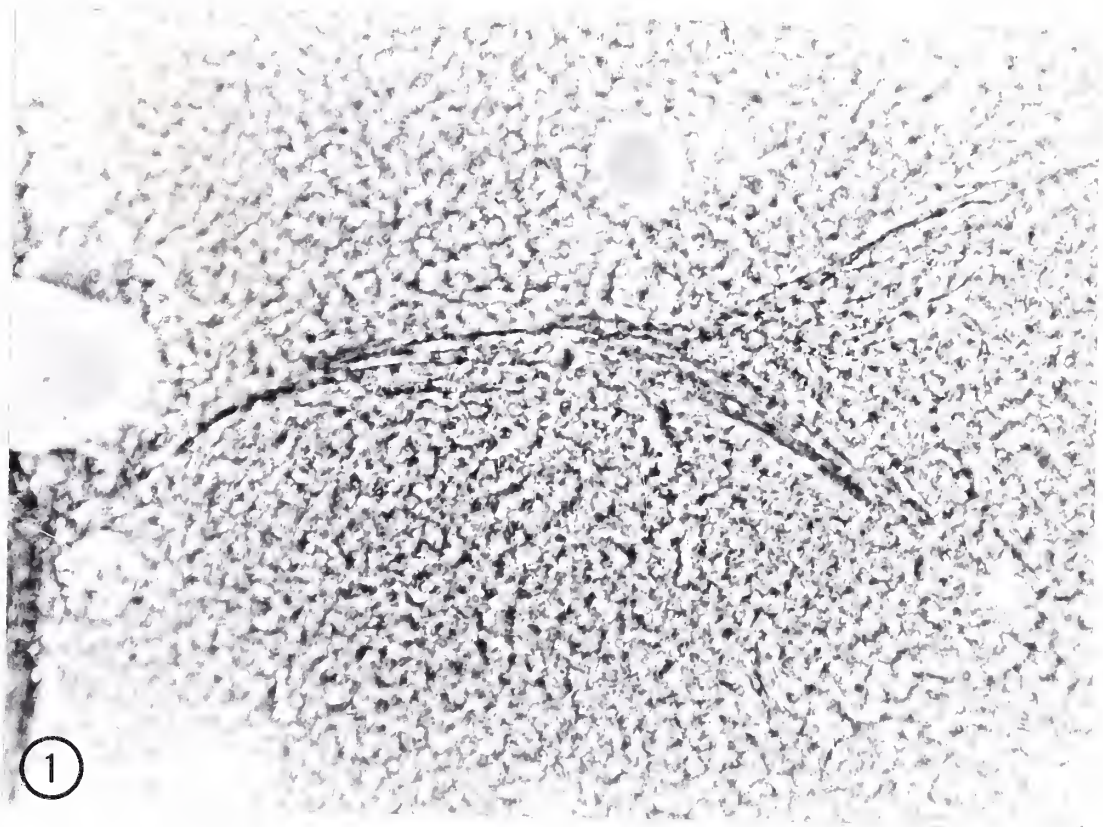
Table 1.

Day	Anatomy of the Organ of Corti	Ultrastructure of Nerve Endings	Myelination of Nerve Fibers	Myelination of Ganglion Cells	Cholinesterase	Efferent Bundle
Newborn	Low columnar hair cells, tunnel unopen, tectorial membrane fused, no spaces of NÜel.	Afferent type present.	None	None	Entire eighth nerve positive. Slightly positive in hair cell region.	Indistinguishable by ACHE activity Questionably present in spiral bundle.
Three Day	No change.	No change.	None	None	No change.	No change.
Five Day	Space forming beneath tectorial membrane.	No change.	Questionable	None	No change.	No change.
Seven Day	Space beneath tectorial membrane enlarging. Spaces of NÜel present, tunnel open to low apical region.	No change.	Many	None	No ACHE in aff. component or spiral ganglion, except in region of spiral bundle. Pos. in spiral lamina, hair cells.	Present as determined by ACHE actovity.
Eight Day	———	———	—	—	———	———
Nine Day	Nearing maturity. Tunnel open.	No change.	Myelinated fibers predominate.	None	Similar to seven days.	Similar to seven days.
Eleven Day	Adult appearance.	Eff. endings present.	No Change.	2-4 layers of satellite cell cytoplasm.	No change.	No change.
Fourteen Day	———	———	—	—	———	———
Twenty-two Day&Adult	Same as eleven day.	No change.	No change.	22D.-10-15 Adult-20	No change.	No change.

Gelatinous Tissue in Middle Ear. (Mikaelian&Ruben,1965)	External Canals (Ibid.)	Preyer Relfex (Kikuchi&Hilding,1965)	Stria Vascularis (Mikaelian&Ruben,1965)	CM (Ibid.)	N_1 (Ibid.)	R_1 (Ibid.)
Present	Closed	Absent	Immature	—	—	—
Present	Closed	Absent	Immature	—	—	—
Present	Closed	Absent	Immature	—	—	—
Present	Closed	Absent	Immature	—	—	—
Resolved	Open	Absent	Mature	Present in 1/2	—	—
Same	Open	Present	Same	Present. in all	1.32 mv.	—
Same	Open	Present	Same	Present	1.25	45mv.
Same	Open	Present	Same	Present	1.10	83
Same	Open	Present	Same	Present	1.00 Adult	162 Adult

Figure 1. Phase contrast micrograph of one day mouse brainstem stained for ACHE activity. Darkly stained lines represent components of the crossed and homolateral efferent bundle. X46.

Figure 2. Phase contrast micrograph of one day mouse spiral ganglion, showing bipolar ganglion cells surrounded by satellite cells containing darkly stained nuclei. X300.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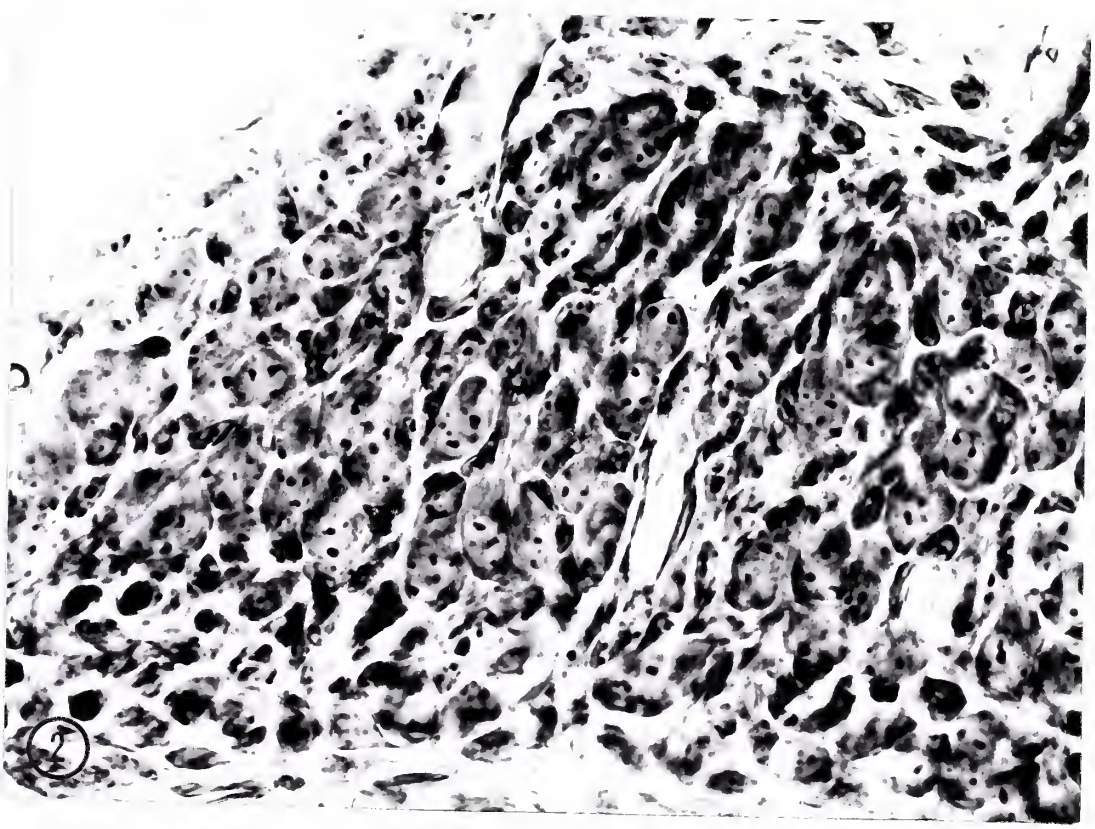
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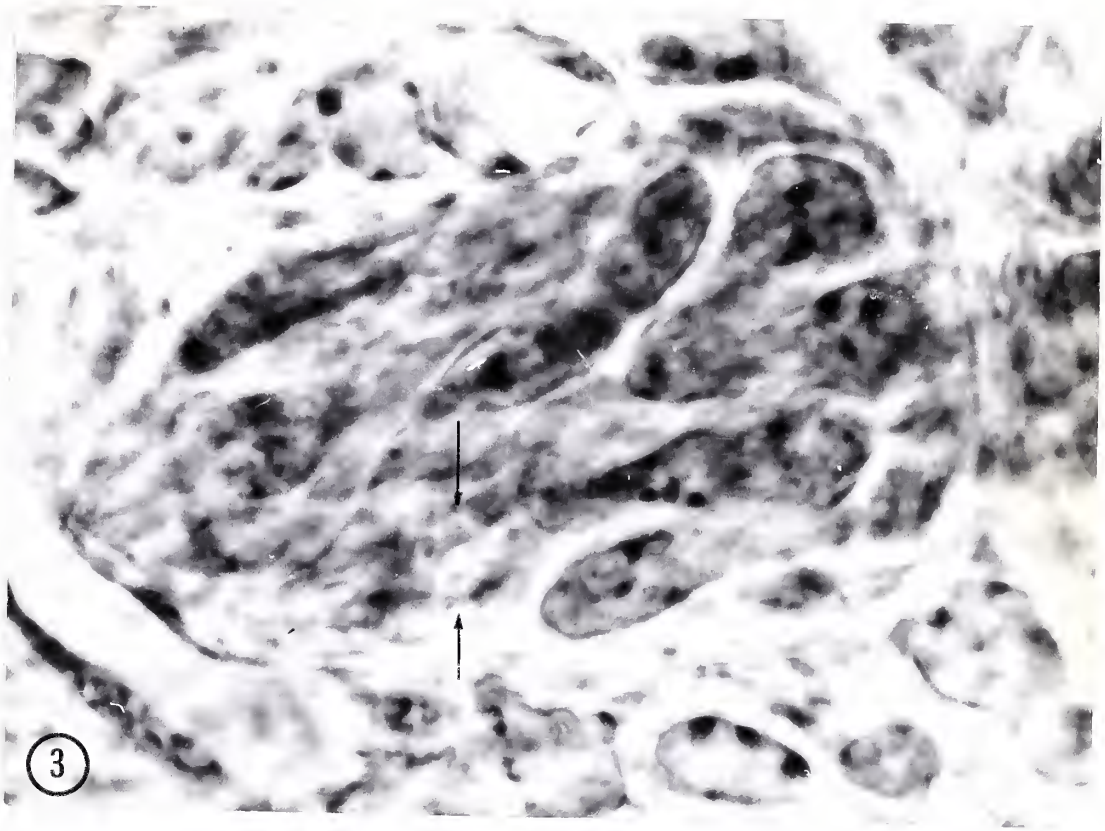
Figure 3. Phase contrast micrograph of one day spiral ganglion showing the lateral portion of that structure. Arrows point to probable efferent fibers cut transversely. X1000.

Figure 4. One day organ of Corti. The hair cells, indicated by arrows, are seen to be of the low columnar type with large basally located nuclei. Pillar cells (PC) are located between the inner and outer hair cells. No fluid spaces are apparent. The tectorial membrane (TM) is poorly visualized but can be seen extending to the hair cells. The spiral vessel (sv) is large. X2100.



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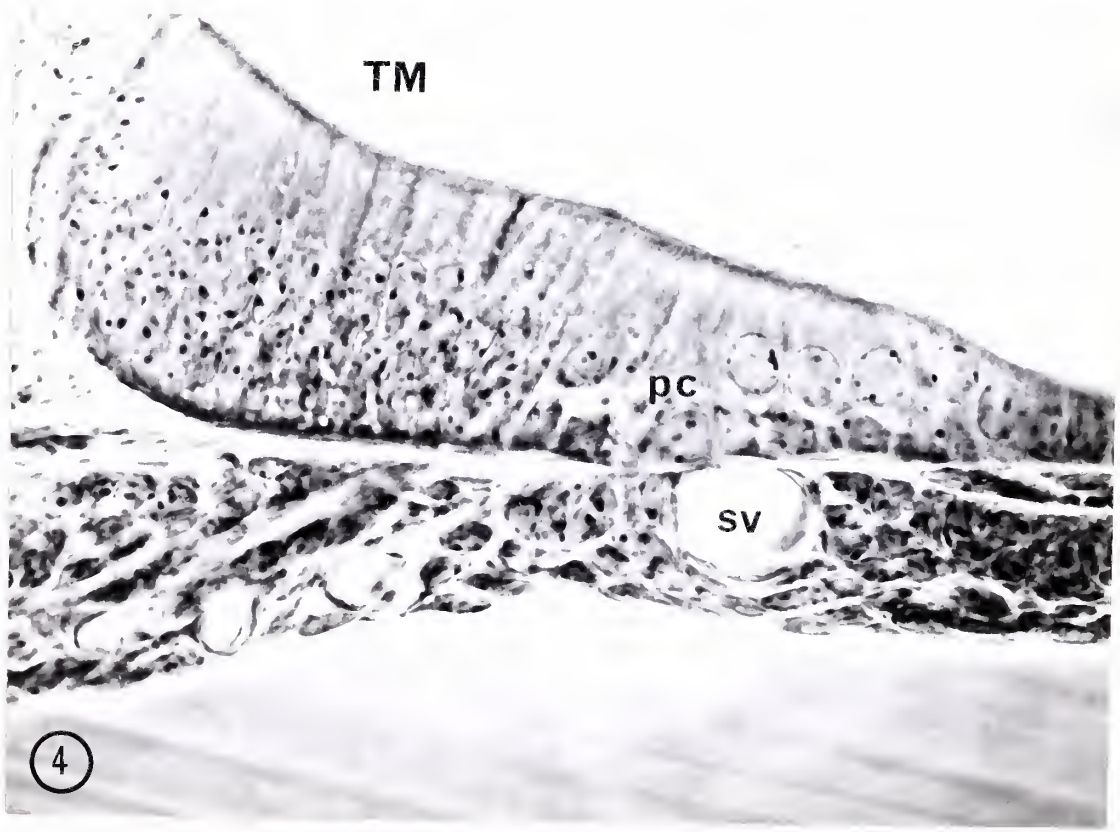

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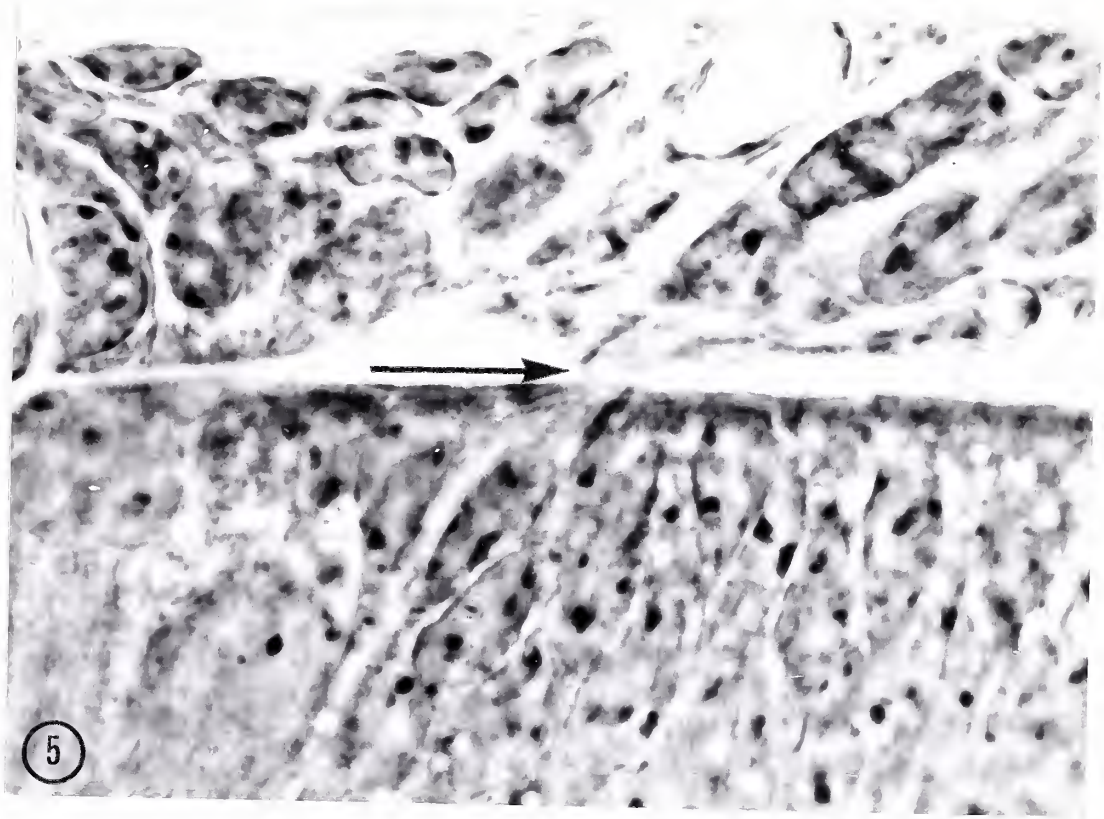
Figure 5, Phase contrast micrograph of one day mouse organ of Corti, in the region of the habenula perforata. Unmyelinated fibers can be observed in the osseous lamina passing through the habenula as indicated by the arrow. X4000.

Figure 6. Phase contrast micrograph of the lateral portion of a three day mouse spiral ganglion. Arrows point to probable efferent fibers cut transversely. X1000.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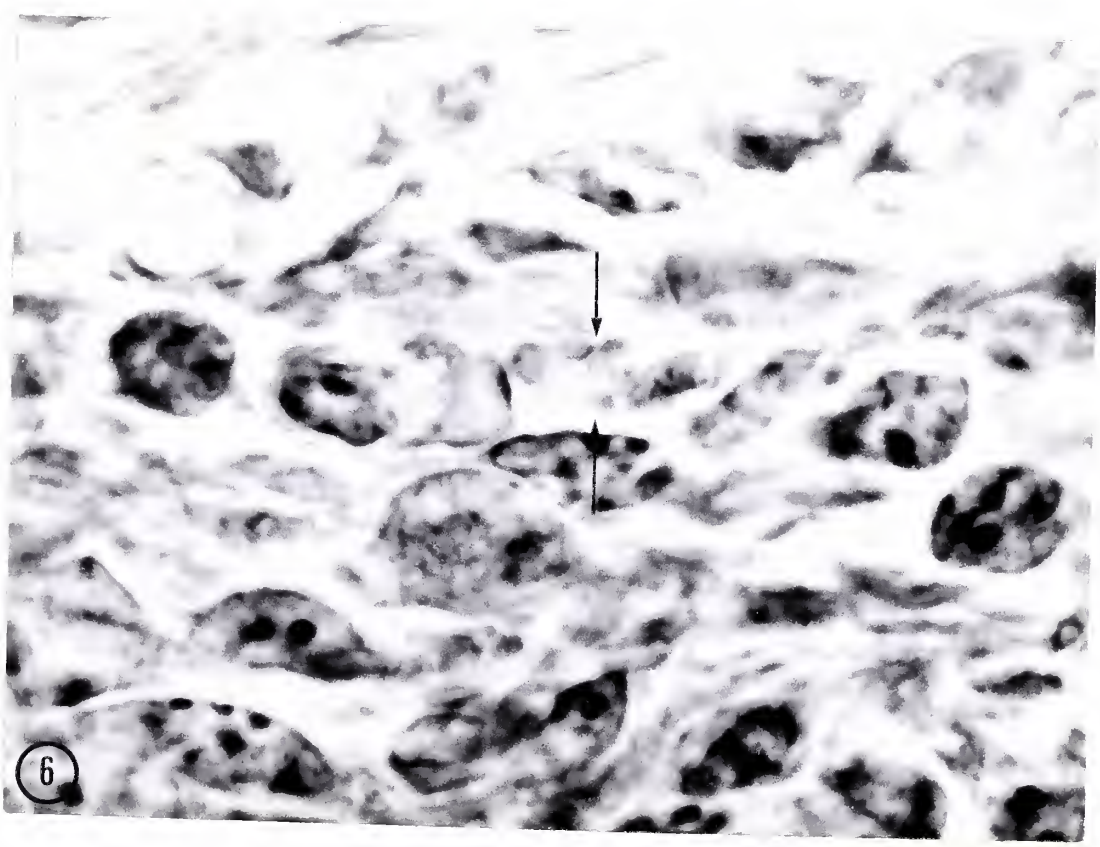
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Figure 7. Phase contrast micrograph of a three day mouse organ of Corti. As in the one day specimen, there are no spaces of Nüel, the pillar cells continue to be apposed, and there is no space beneath the tectorial membrane. Nerve fibers can be seen passing through the habenula as demonstrated by the arrow. X2200.

Figure 8. Phase contrast micrograph of a five day mouse organ of Corti. As denoted by the (X),a space is present beneath the tectorial membrane. X1500.

7

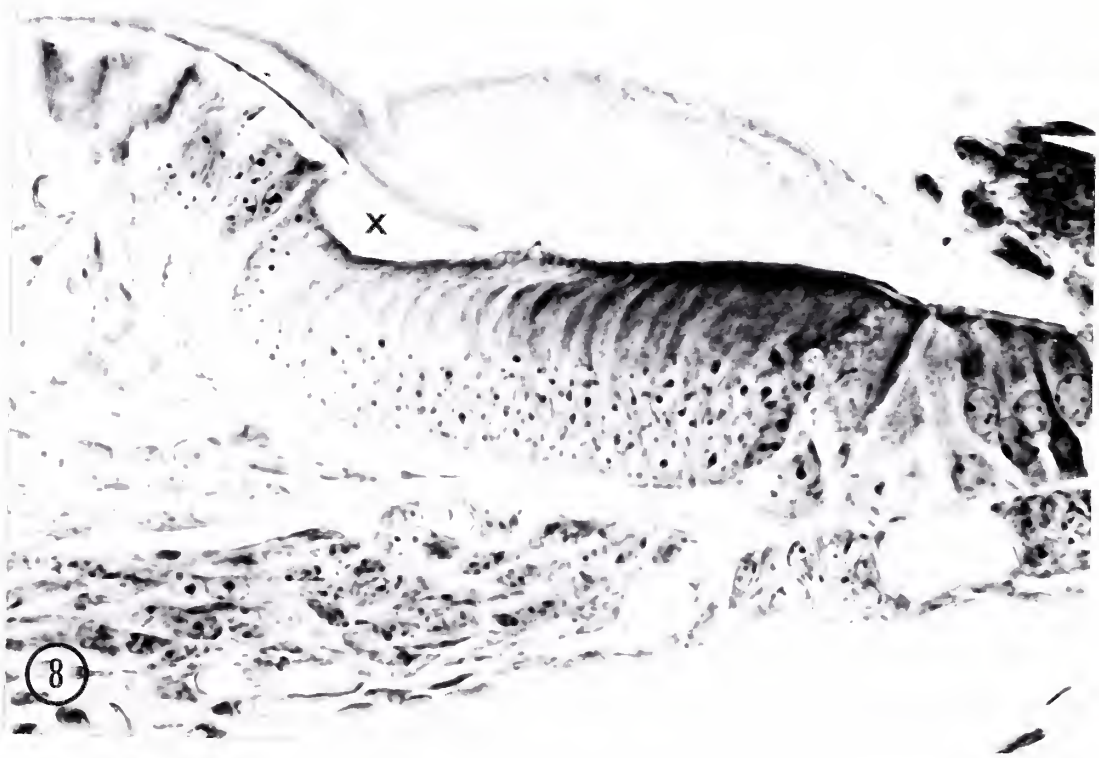
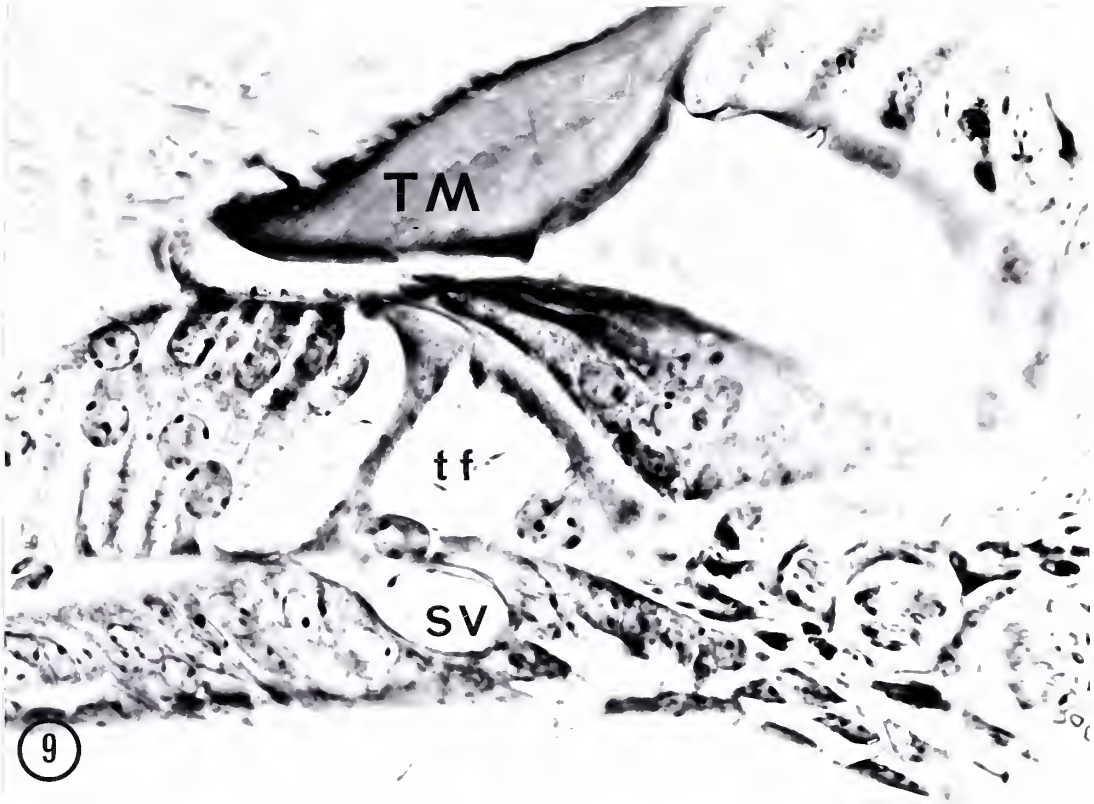

X
8

Figure 9. Phase contrast micrograph of a seven day mouse organ of Corti. Note the increasingly large space between the tectorial membrane (TM). The open tunnel of Corti with tunnel fibers (tf) is well apparent. The spiral vessel (SV) is now less prominent. X2200.

Figure 10. Phase contrast micrograph of a nine day mouse organ of Corti stained for ACHE activity. Intense activity is limited to the efferent fibers (EF) in the osseous lamina and to the region beneath the hair cells (HC). X2100.



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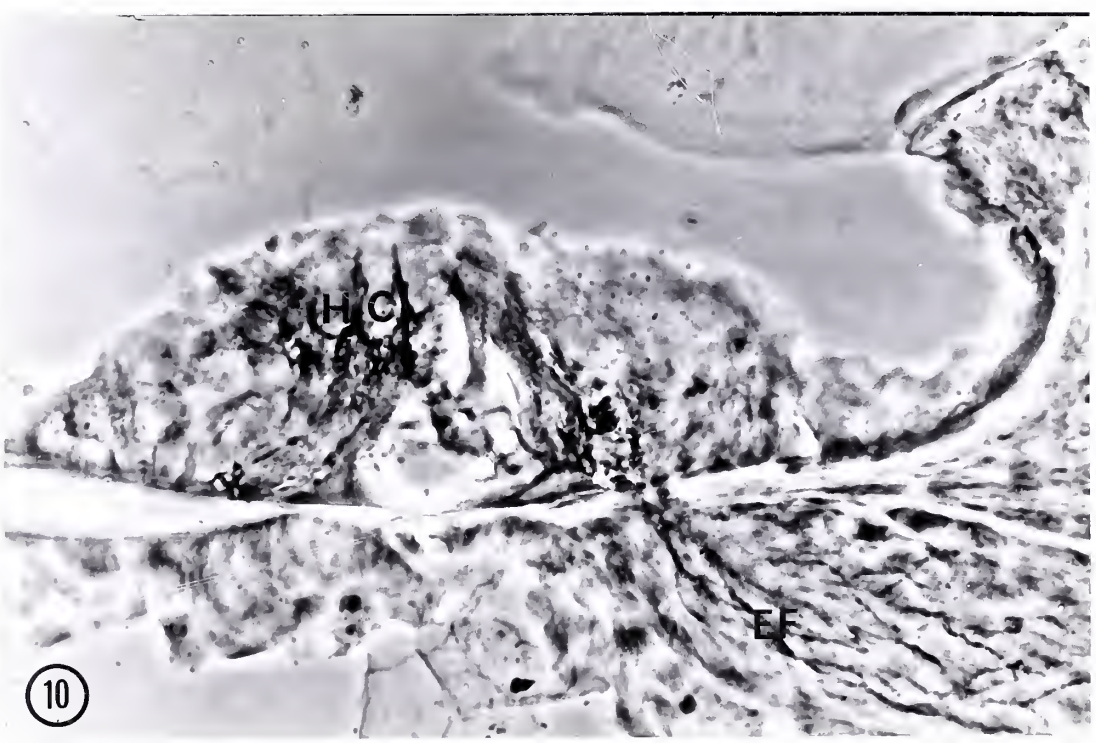

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Figure 11. Phase contrast micrograph of a nine day mouse organ of Corti. The organ at this stage is reaching the appearance of the organ in the adult animal. Myelinated fibers (NF) are present in the osseous lamina. The tunnel of Corti is open throughout and fluid spaces (spaces of Nüel) (SN) are present. X2000.

Figure 12. Phase contrast micrograph of an eleven day mouse organ of Corti. Essentially an adult configuration. Note myelinated nerve fibers, cuboidal cells (cc) beneath the tectorial membrane (TM), and one inner (IHC) and three outer hair cells (OHC) with attached hairs. X2000.

11

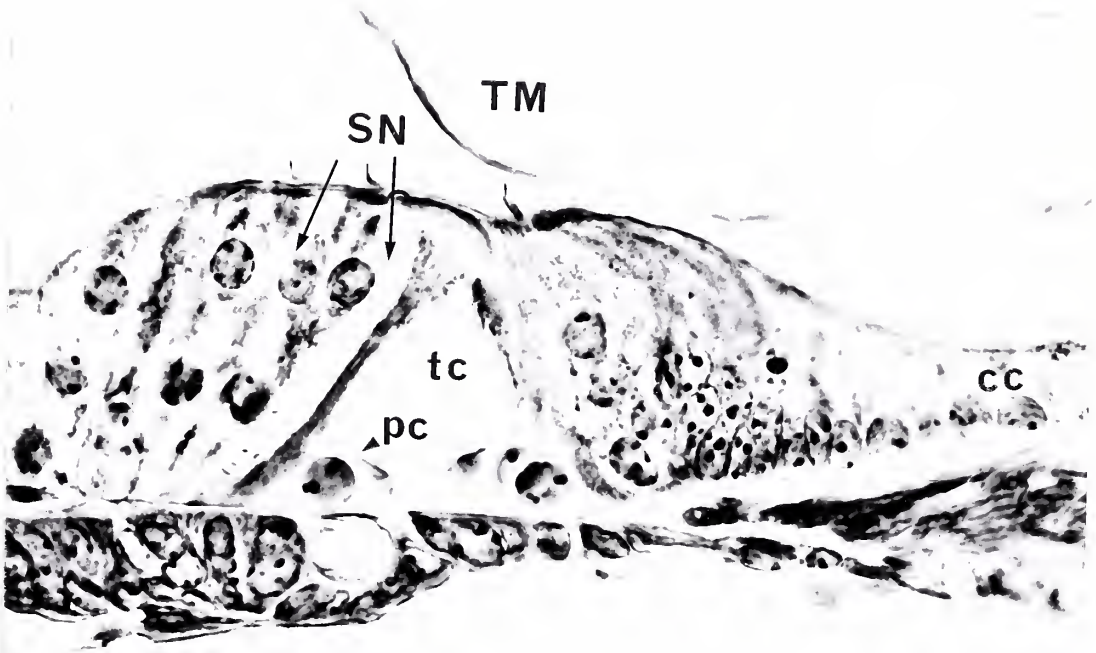

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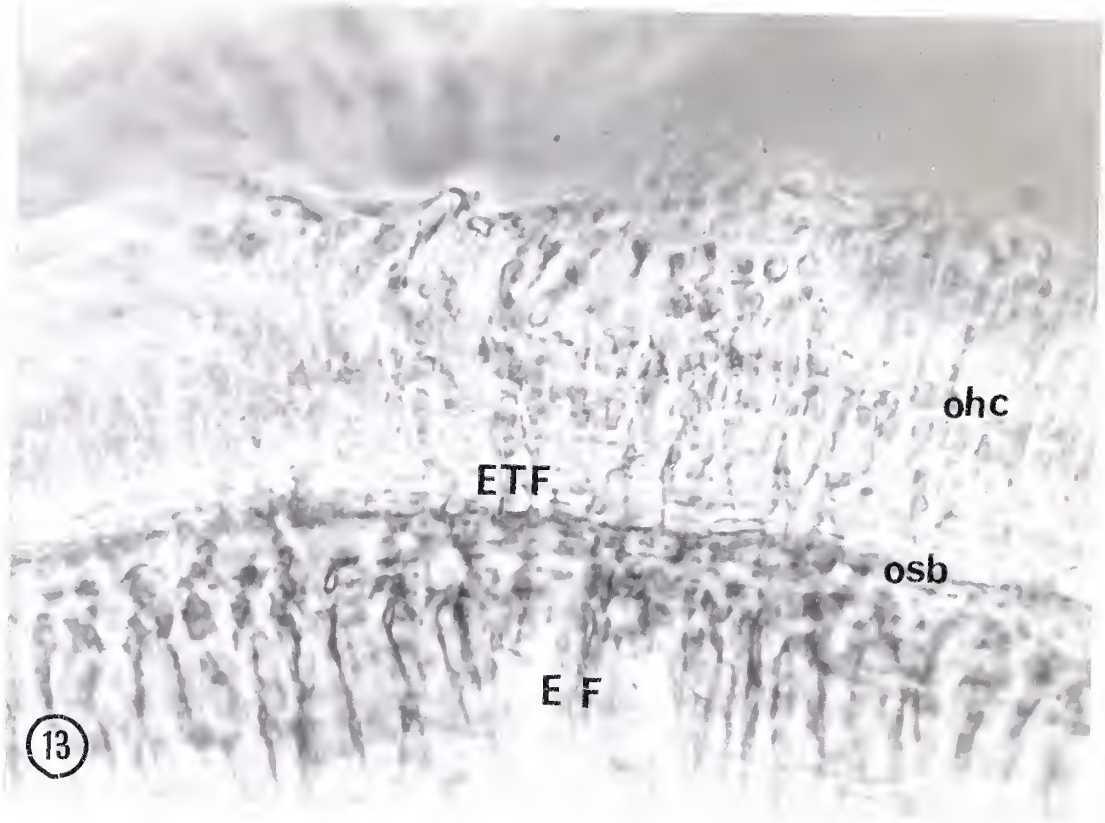
Figure 13. Phase contrast micrograph of a surface preparation of the adult organ of Corti stained for ACHE activity. Note the efferent fibers in the region of the osseous lamina (EF), the outer spiral bundle of efferent fibers (OSB), efferent tunnel fibers (ETF) crossing the tunnel of Corti, and the region of the outer hair cells (OHC). X850.

Figure 14. Phase contrast micrograph of an adult organ of Corti, essentially unchanged from that seen in Fig. 13. X1700.


ohc
ETF
osb
E F
13

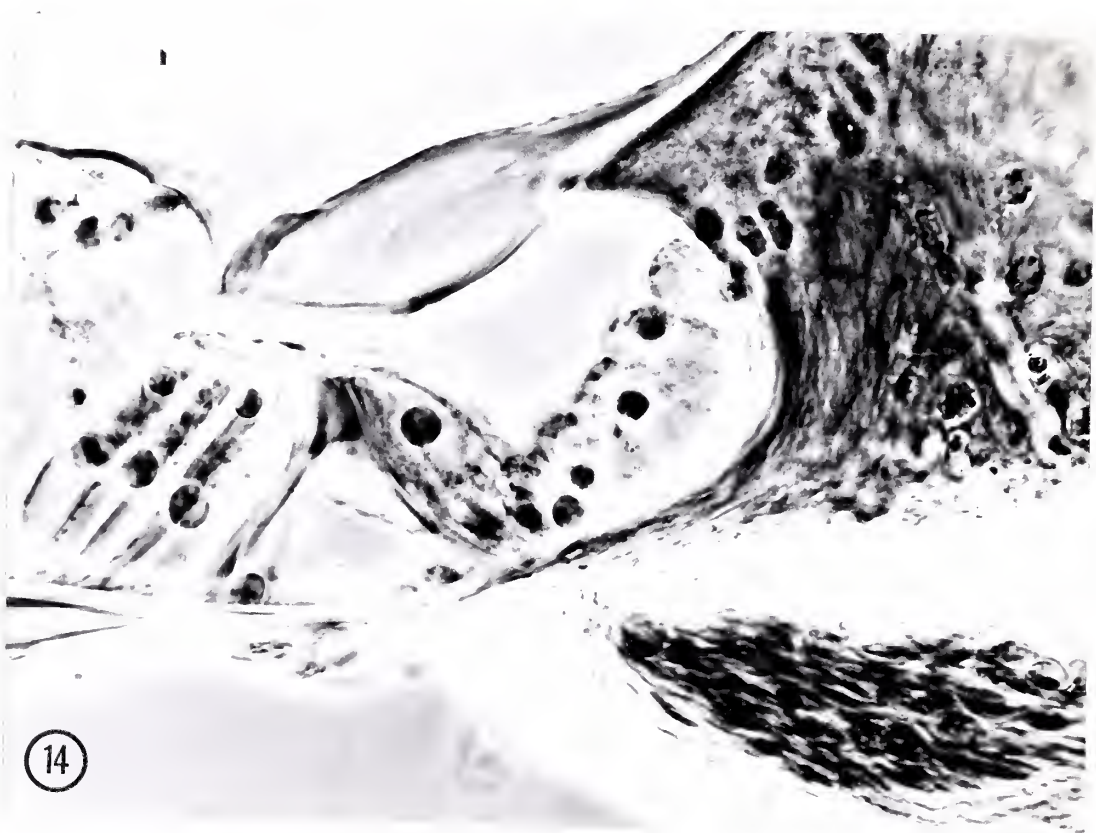
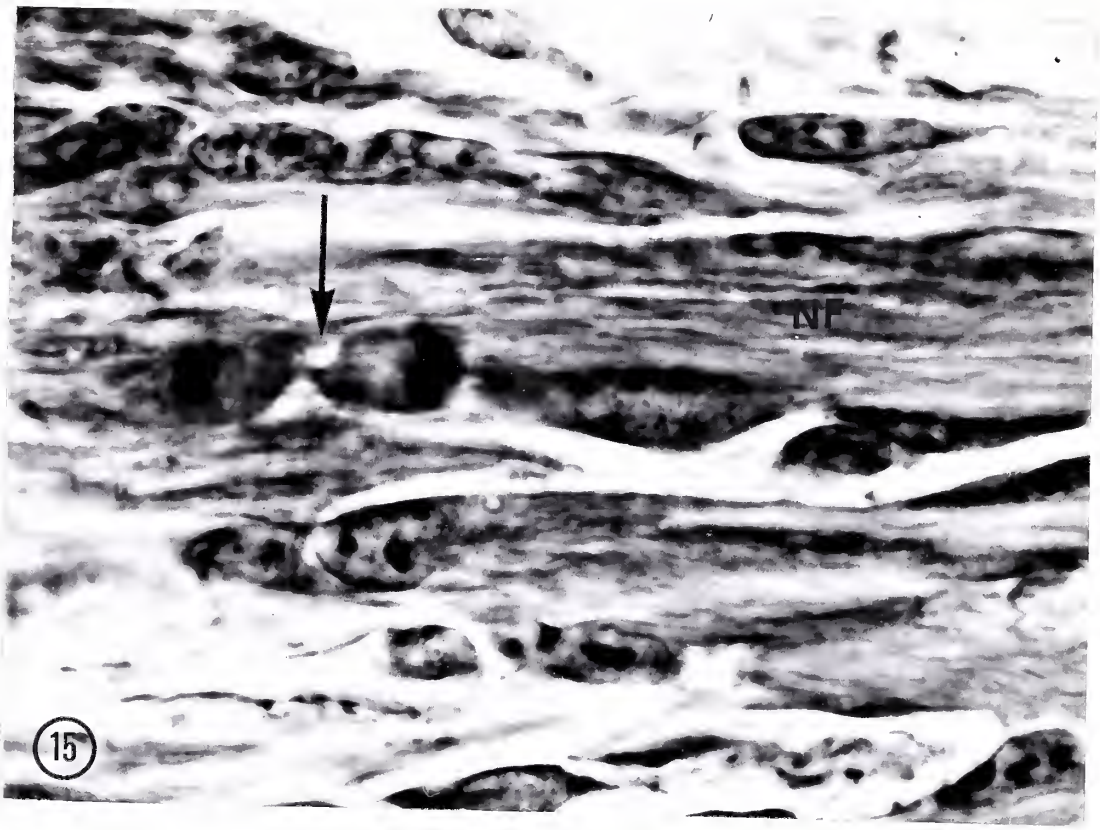

14

Figure 15. Phase contrast micrograph of the eighth nerve in the modiolus of a newborn mouse cochlea. Note the lack of dense lines outlining nerve fibers (NF). An arrow points to a Schwann cell mitotic figure. Such figures are seen with some frequency in young mice. X800.

Figure 16. A higher magnification of figure 18. Note the Golgi apparatus (GA) in the satellite cell cytoplasm and the rough ER in the perinuclear region. Peripheral satellite cell mitochondria are generally spherical (M). X8800.


NF
15

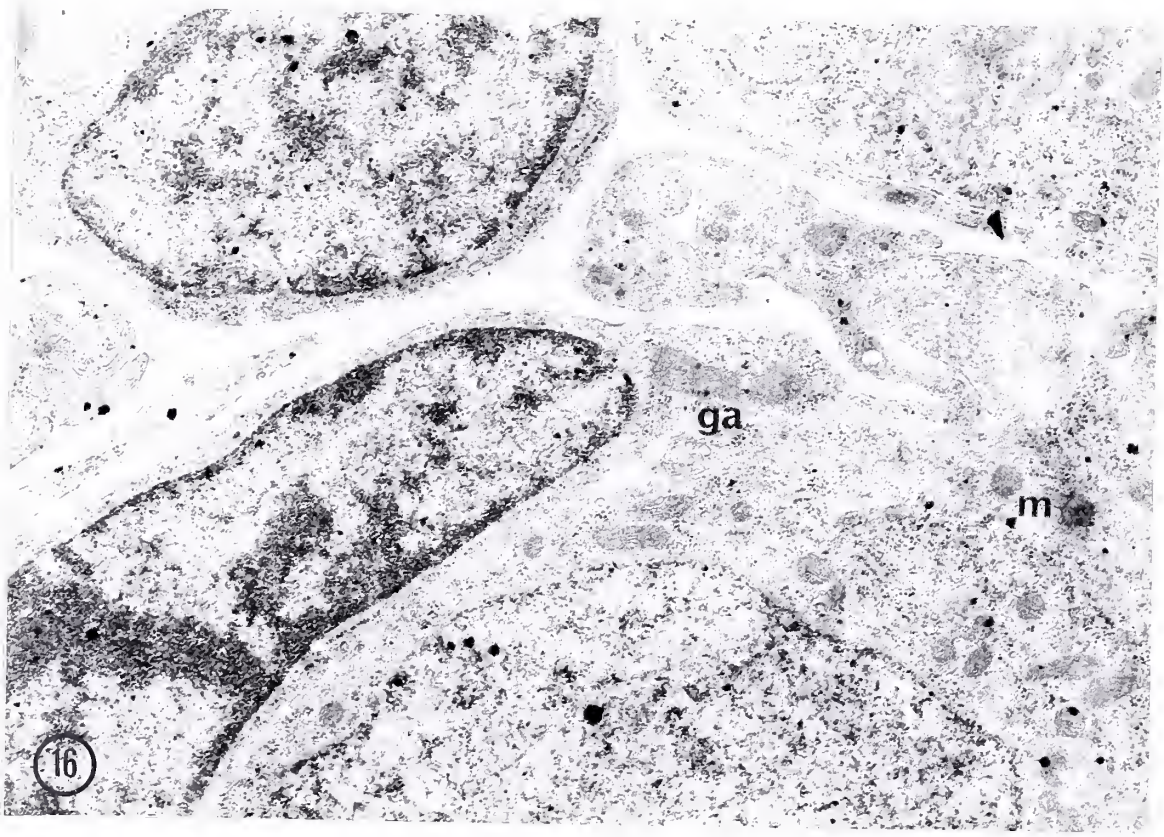
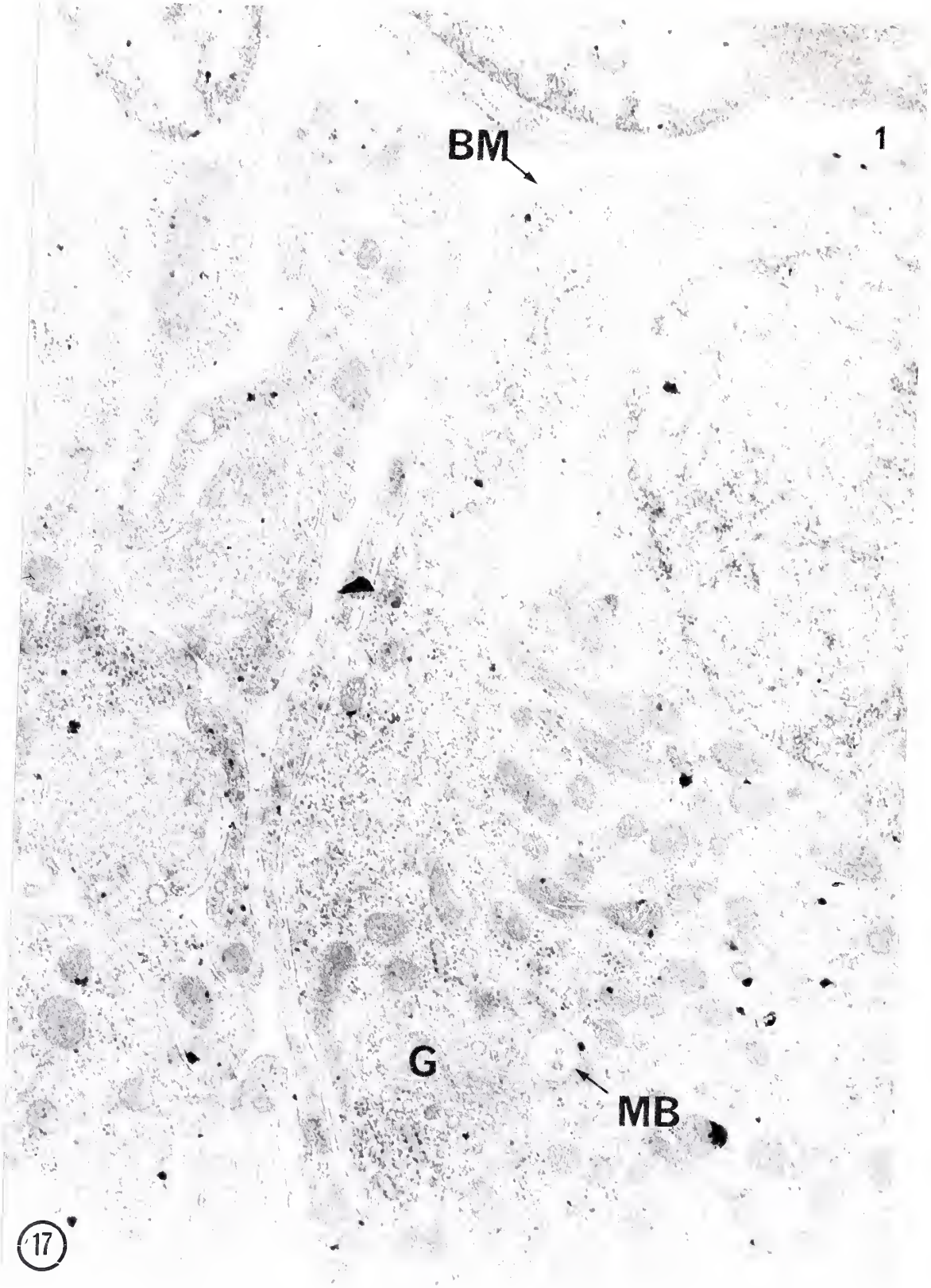

ga
m
16

Figure 17. Electron micrograph of a one day mouse spiral ganglion. Note the Golgi apparatus (G) and multivesicular body (MB) in the bipolar ganglion cell cytoplasm. A discontinuity in the satellite cell cytoplasm is apparent at (1). The basement membrane (BM) covering the satellite cell cytoplasm can be seen engulfing an adjacent neuron. X10,000.



17

Figure 18. Electron micrograph of a one day mouse spiral ganglion. Two neurons are present, one showing an eccentric nucleolus (NL) in the nucleus (N). Note the cylindrical mitochondria (M) and rough endoplasmic reticulum (ER) in the above cells. Each cell is ensheathed by a single layer of satellite cell cytoplasm (C). Note the discontinuity of such cytoplasm at (1). A "T" formation is shown at (2). A Schwann cell (SC) is seen encasing a large neuron (NE). X5000.

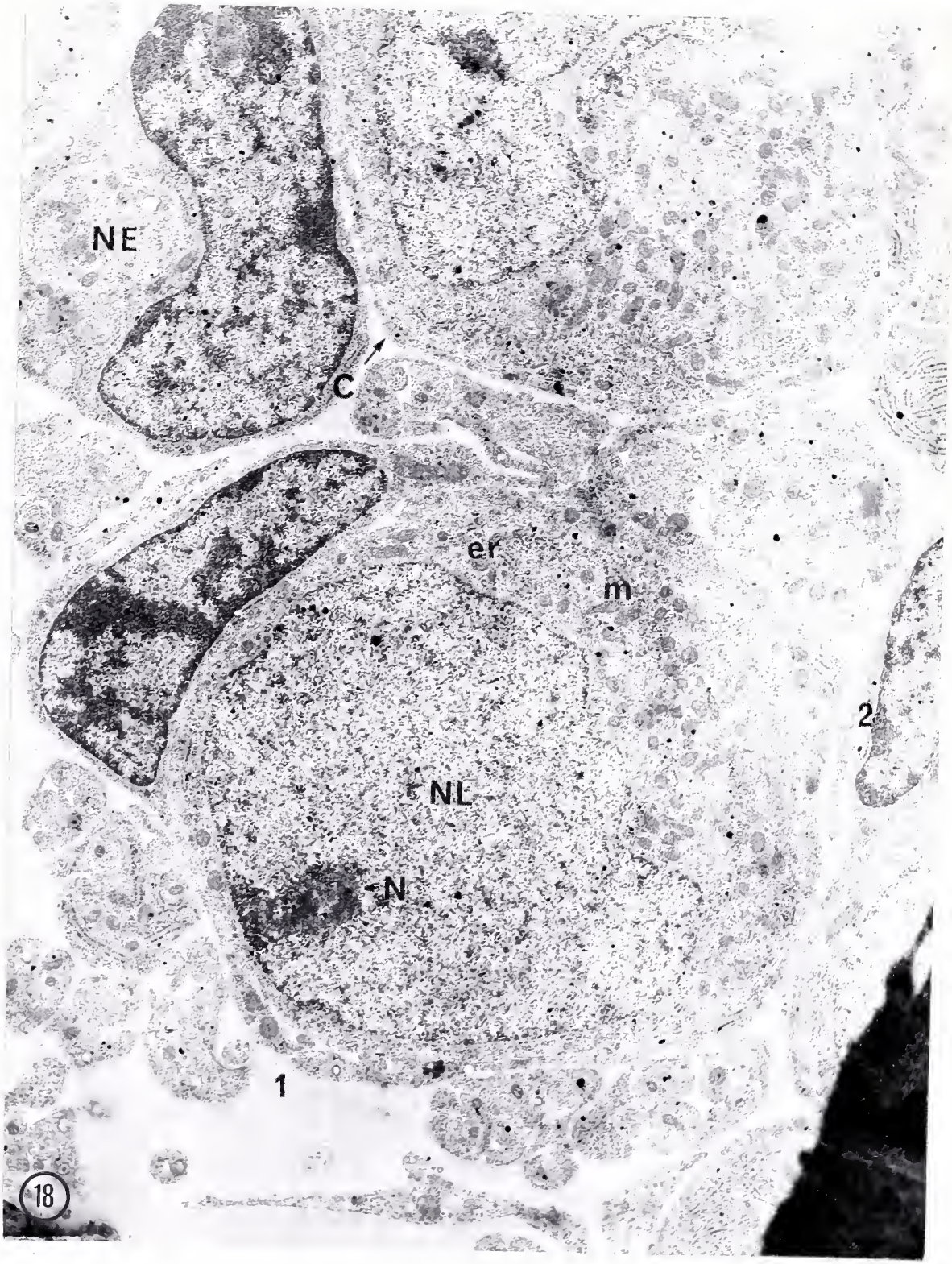
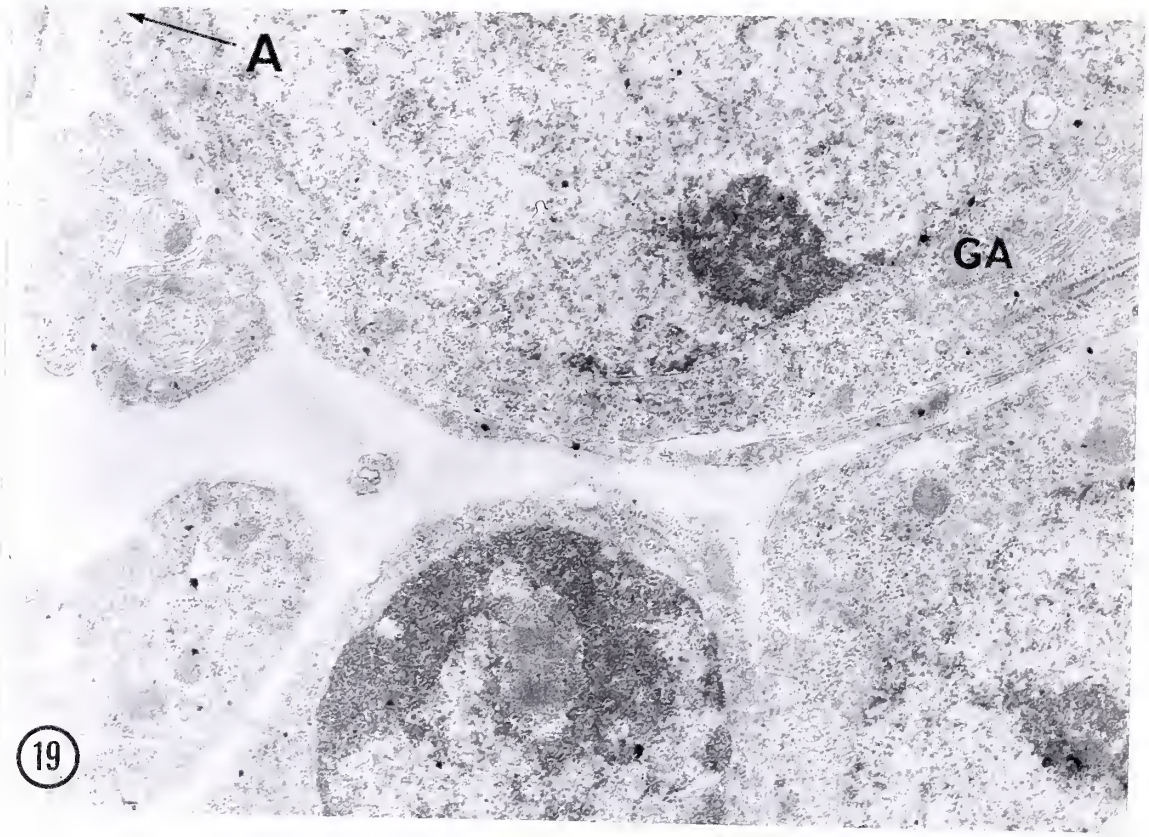

NE
C
er
m
2
NL
N
1
18

Figure 19. Electron micrograph of a newborn mouse spiral ganglion. Note the well demonstrated Golgi apparatus (GA) and the discontinuity in the satellite cell cytoplasm at (A). X6000.

Figure 20. Electron micrograph of a newborn mouse spiral ganglion. Of particular interest is the overlap in satellite cell cytoplasm apparent at (O). The great majority of bipolar neuron cell bodies at this stage show no such overlap. X7000.


A
GA
19

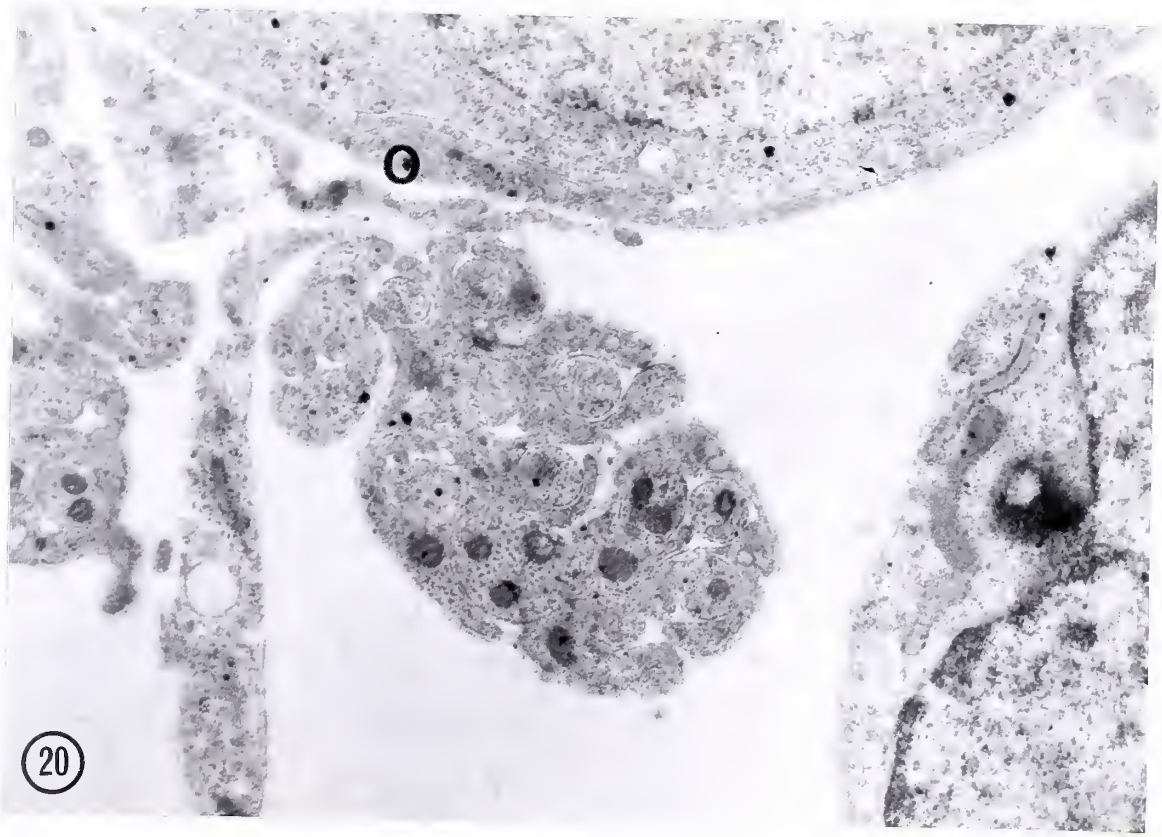

O
20

Figure 21. Electron micrograph of a one day mouse spiral ganglion. Neurotubules (nt), neurofilaments (nf), and characteristic mitochondria (m) with single longitudinal cristae are easily seen. A classic structure similar to that published by Geren (1954) showing a Schwann cell engulfing a nerve fiber is noted in the upper left hand corner of the photograph. In the lower portion of the figure are many nerve fibers surrounded by several Schwann cell processes. At (A), arrows point to three nerve fibers enveloped by a continuous sheath of Schwann cell cytoplasm. At (ER), a dense type of endoplasmic reticulum is seen . X9000.

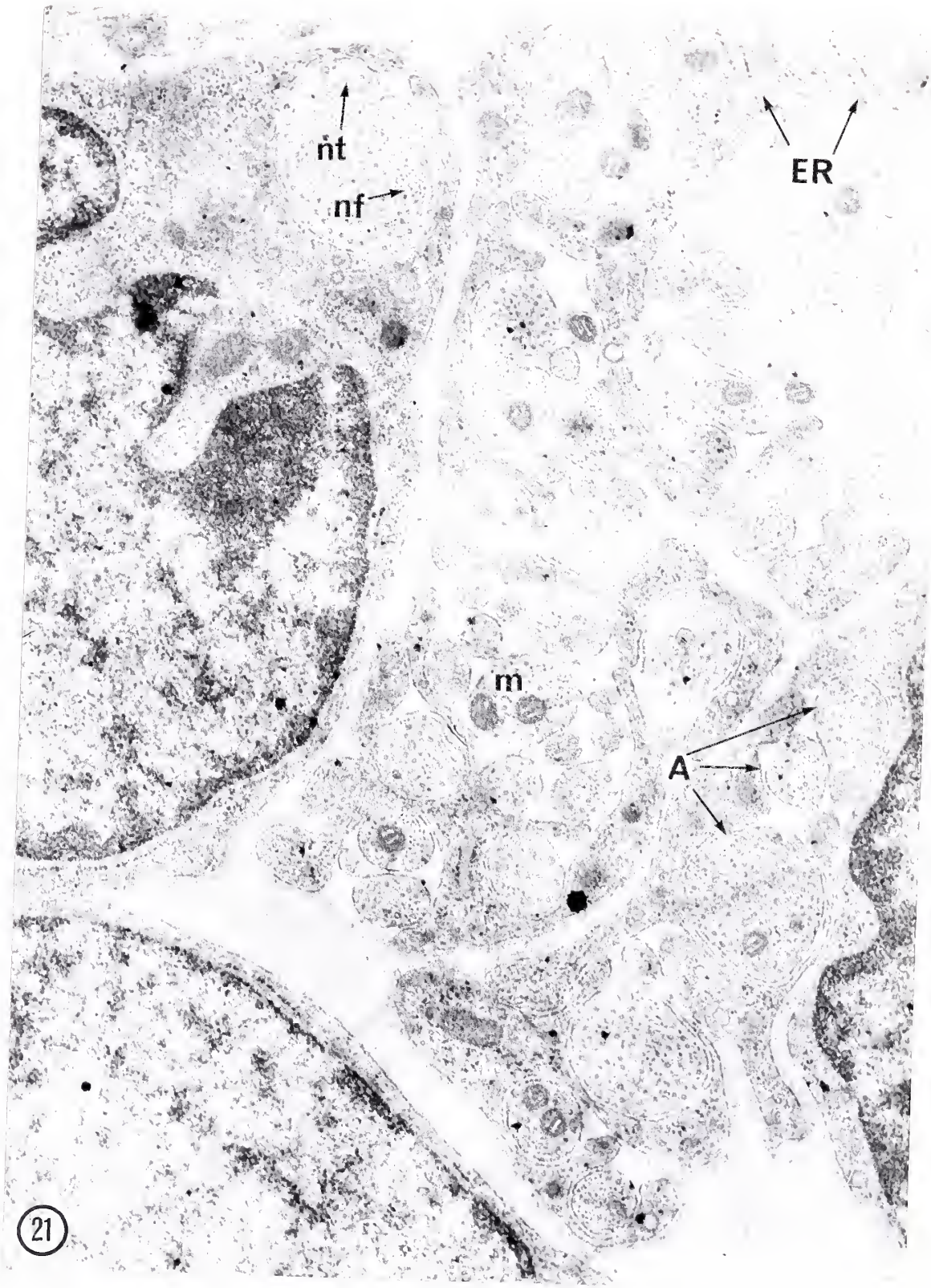

nt
nf
ER
m
A
21

Figure 22. Electron micrograph of a three day mouse spiral ganglion; of note is the tongue (T) of satellite cell cytoplasm inserted beneath the main body of that cytoplasm and the bipolar ganglion cell. A pseudopod-like (P) structure is seen branchnig from the satellite cell cytoplasm encasing the upper neuron. In that same cell, a redundant process of cytoplasm is seen to be inserting itself between the main body of satellite cell cytoplasm and the neuron (rp). X7000.

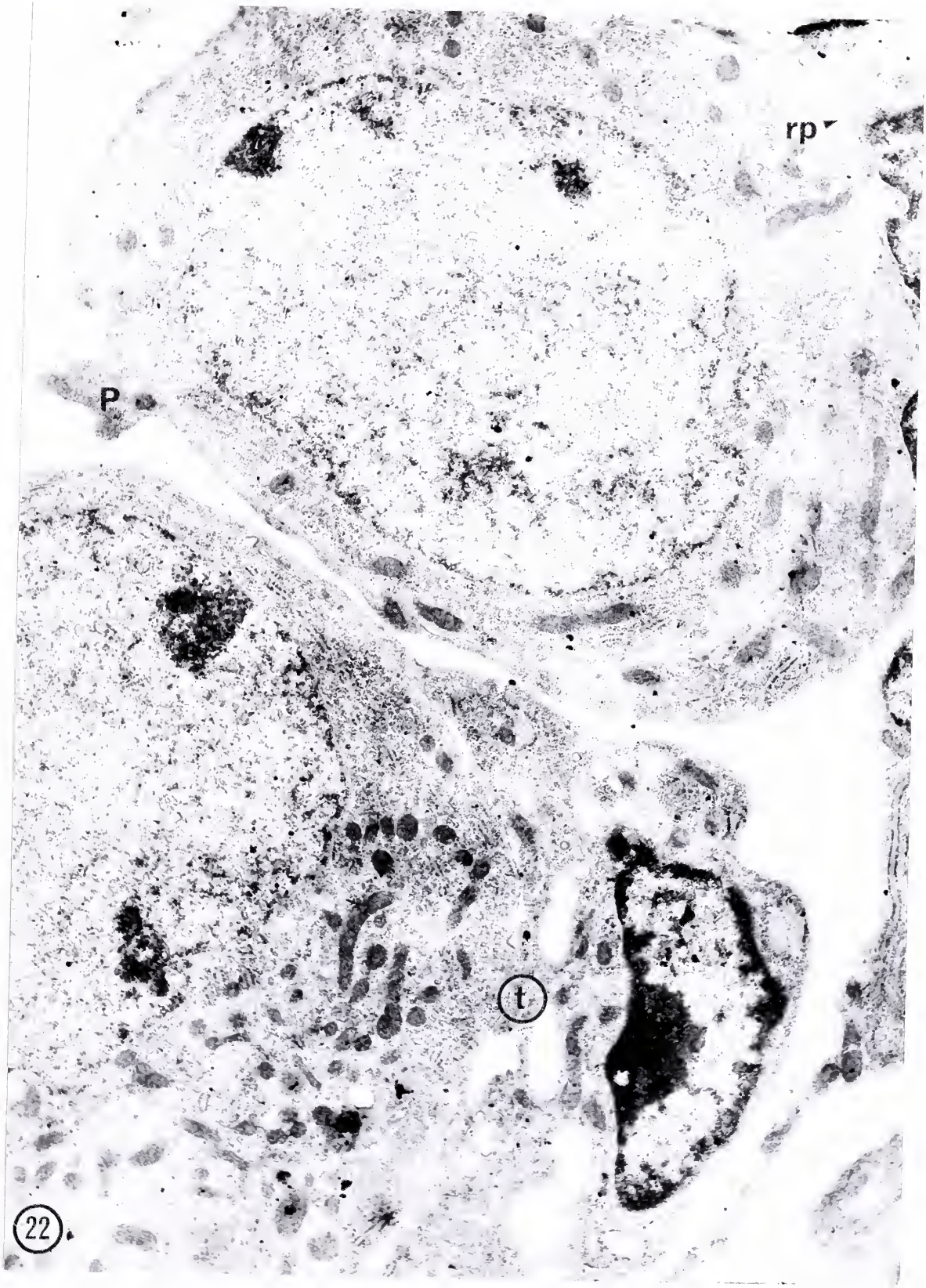
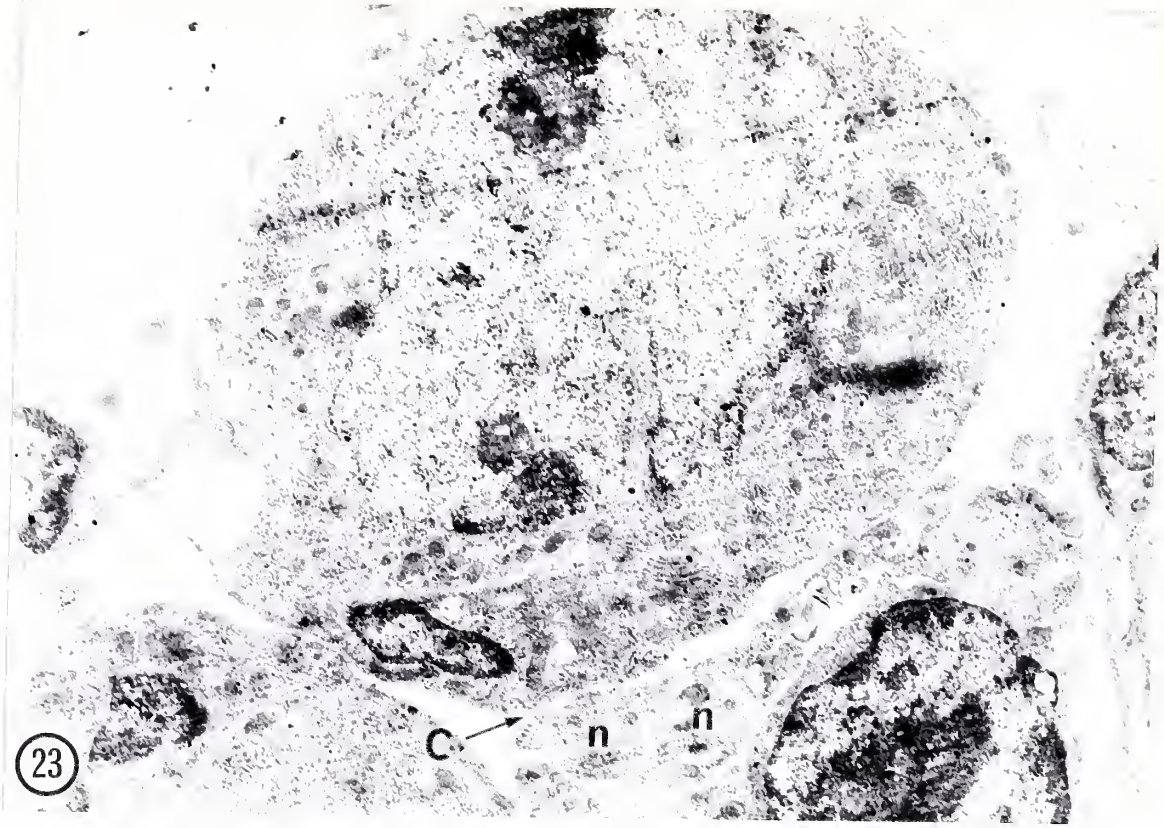

rp
P
t
22

Figure 23. Electron micrograph of a three day mouse spiral ganglion. At (C), a connection is noted between the satellite cell cytoplasm and cytoplasm enveloping two nerve fibers (n). X4200.

Figure 24. Electron micrograph of a three day mouse spiral ganglion. At (O), a single branching offshoot is noted. The significance of such a process is unknown but it could be that it reaches to ensheath another cell body or nerve fiber. X5000.



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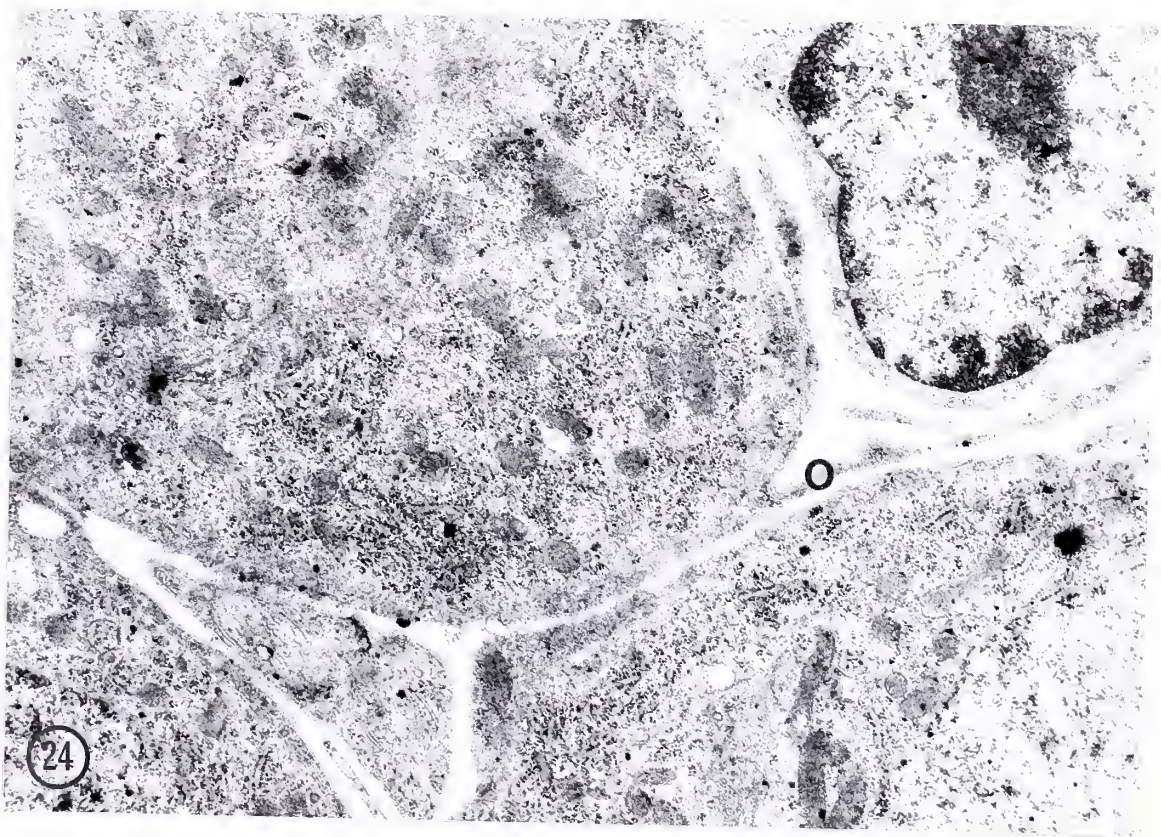
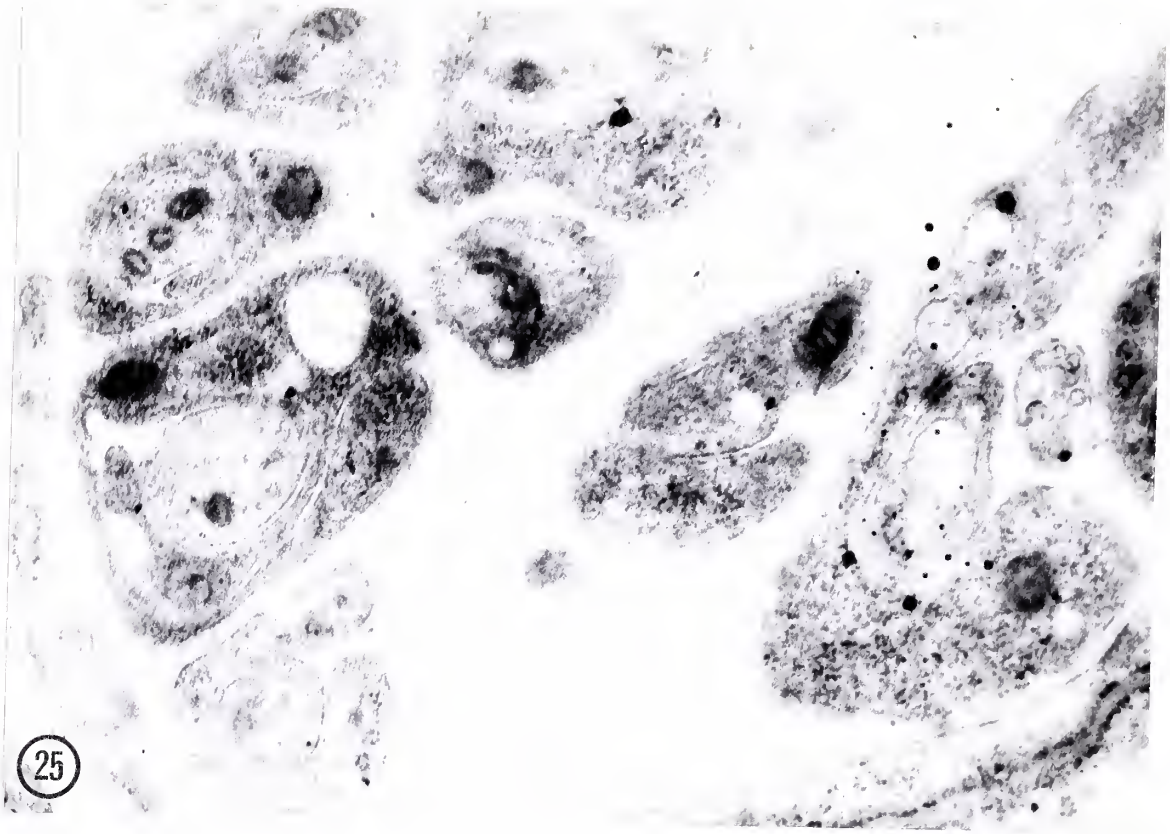


24

Figure 25. Electron micrograph of a three day mouse spiral ganglion. On the left in the photograph, a slightly more extensively ensheathed nerve fiber is noted. A desmosome is seen at (D). Such structures are rare in the process of myelination. X9000.

Figure 26. Phase contrast micrograph of a five day mouse cochlea showing an isolated segment of myelination along a nerve fiber (N). X 750.



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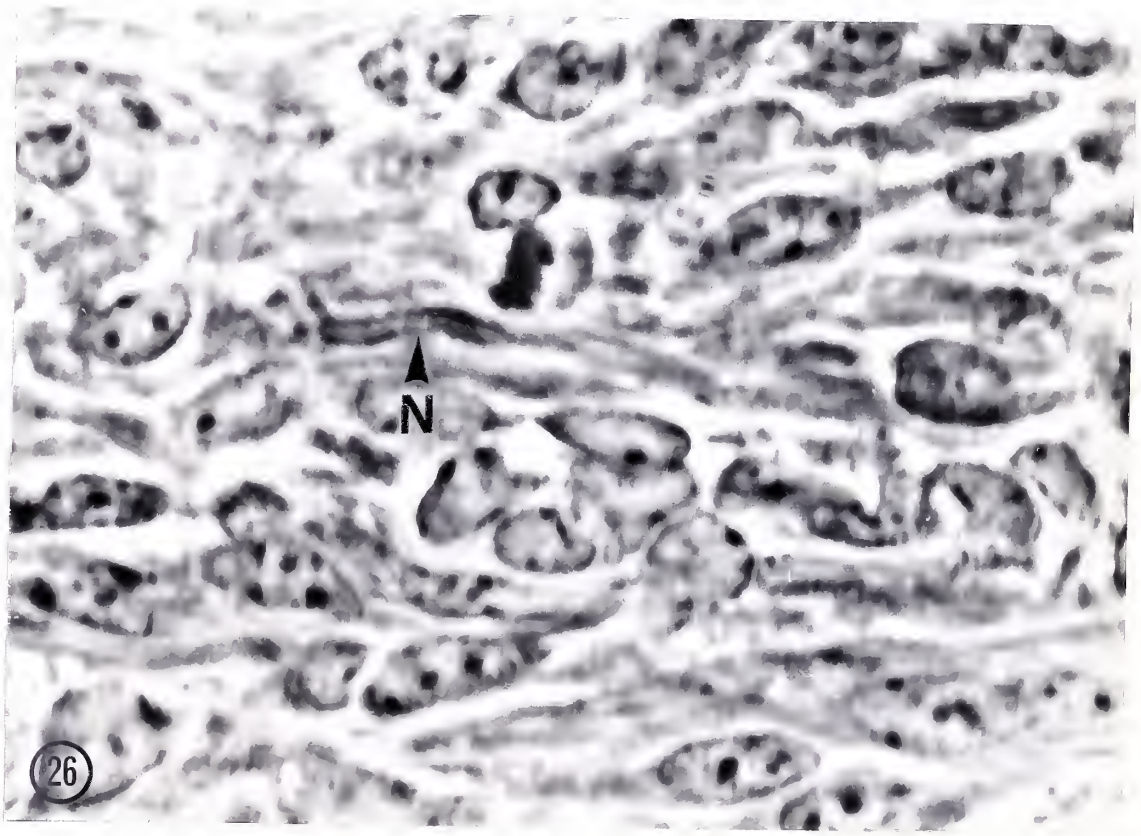
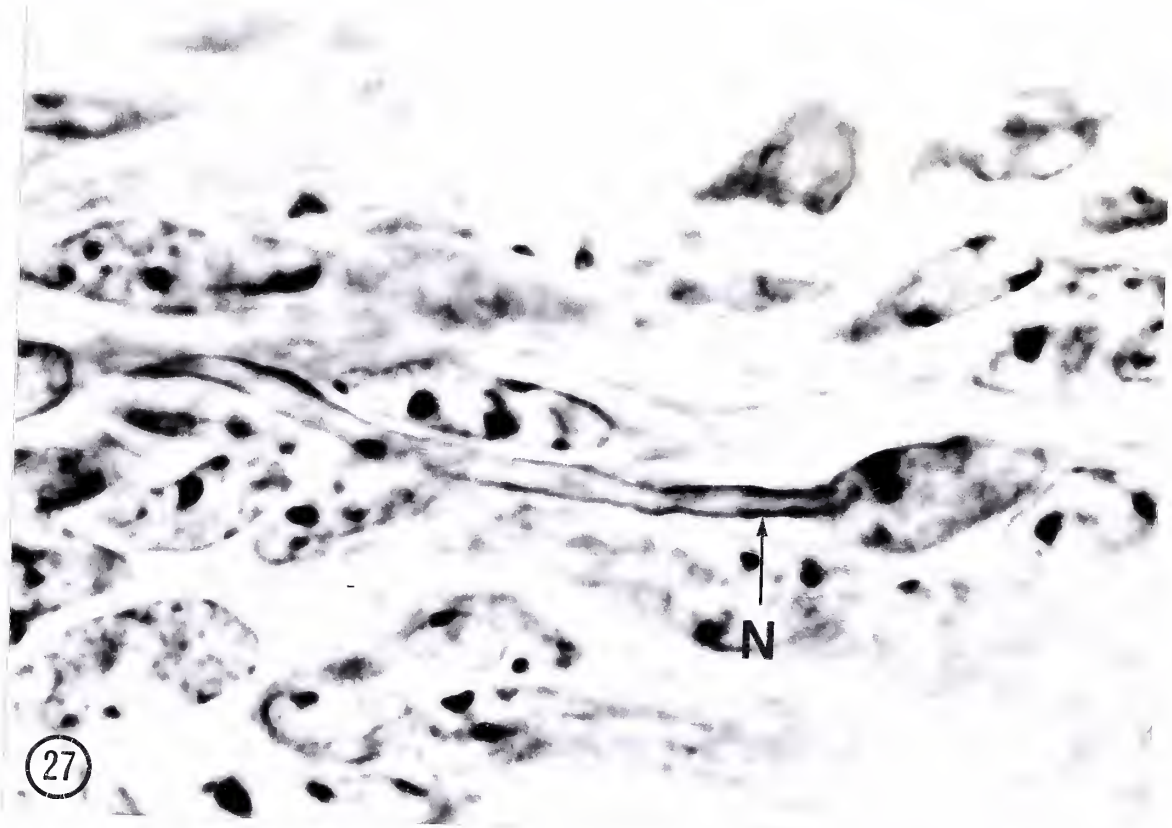


26

Figure 27. Phase contrast micrograph of a five day mouse cochlea taken in the region of the osseous spiral lamina. Note the one isolated myelinated nerve fiber (N). X1500.

Figure 28. Phase contrast micrograph of a five day mouse spiral ganglion. The two arrows point to two satellite cell nuclei about one neuron cell body. X1000.



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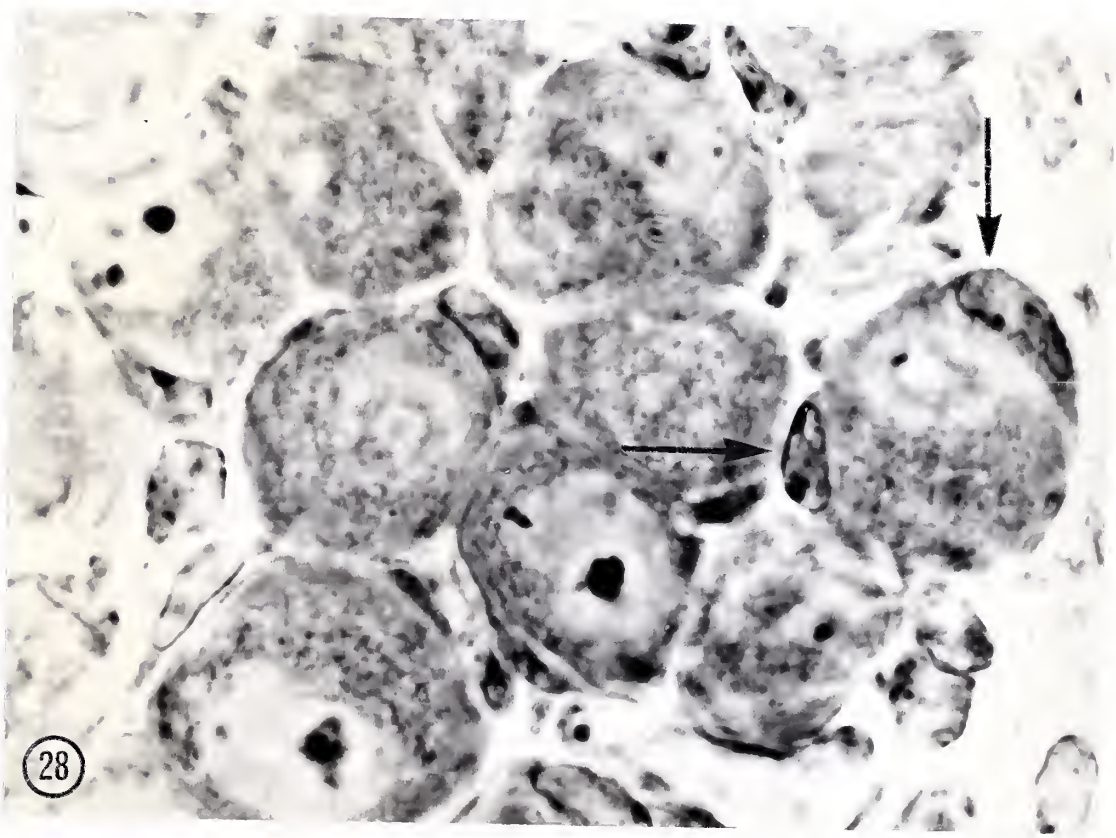
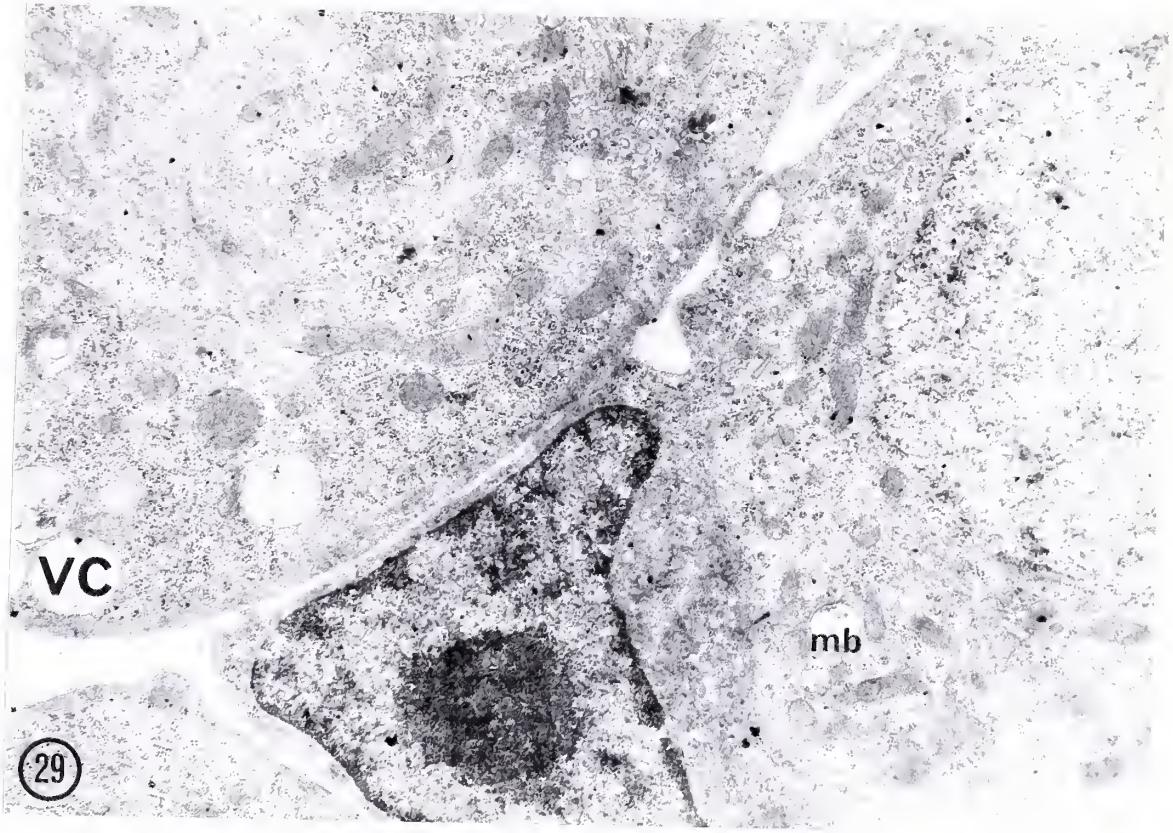


28

Figure 29. Electron micrograph of a five day mouse spiral ganglion. Note the multivesicular bodies (mb) in the cell on the right. In the adjacent cell, four larger vacuoles (VC), having finely granulated contents, are distinctly different from the multivesicular bodies. X7000.

Figure 30. Electron micrograph of a five day mouse spiral ganglion. An obliquely sectioned nerve fiber is shown encircled by one to two layers of cytoplasm. X18,000/


VC
mb
29

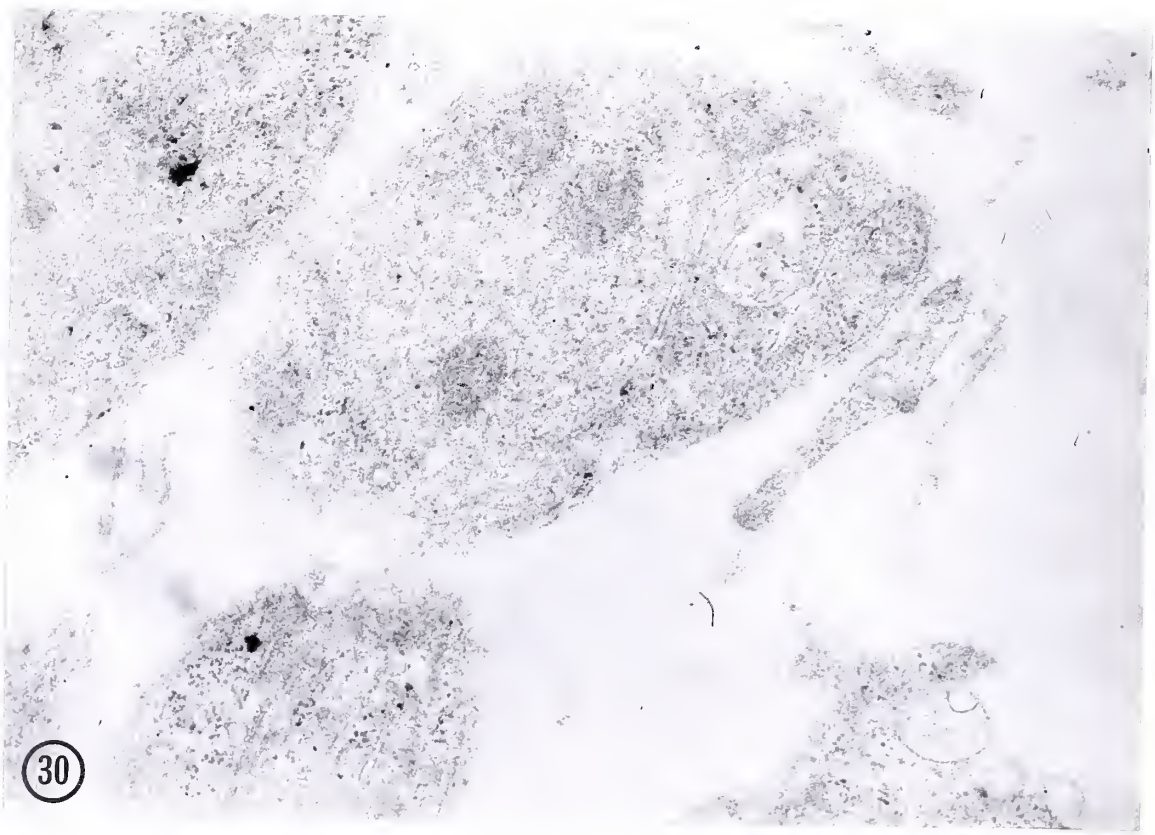
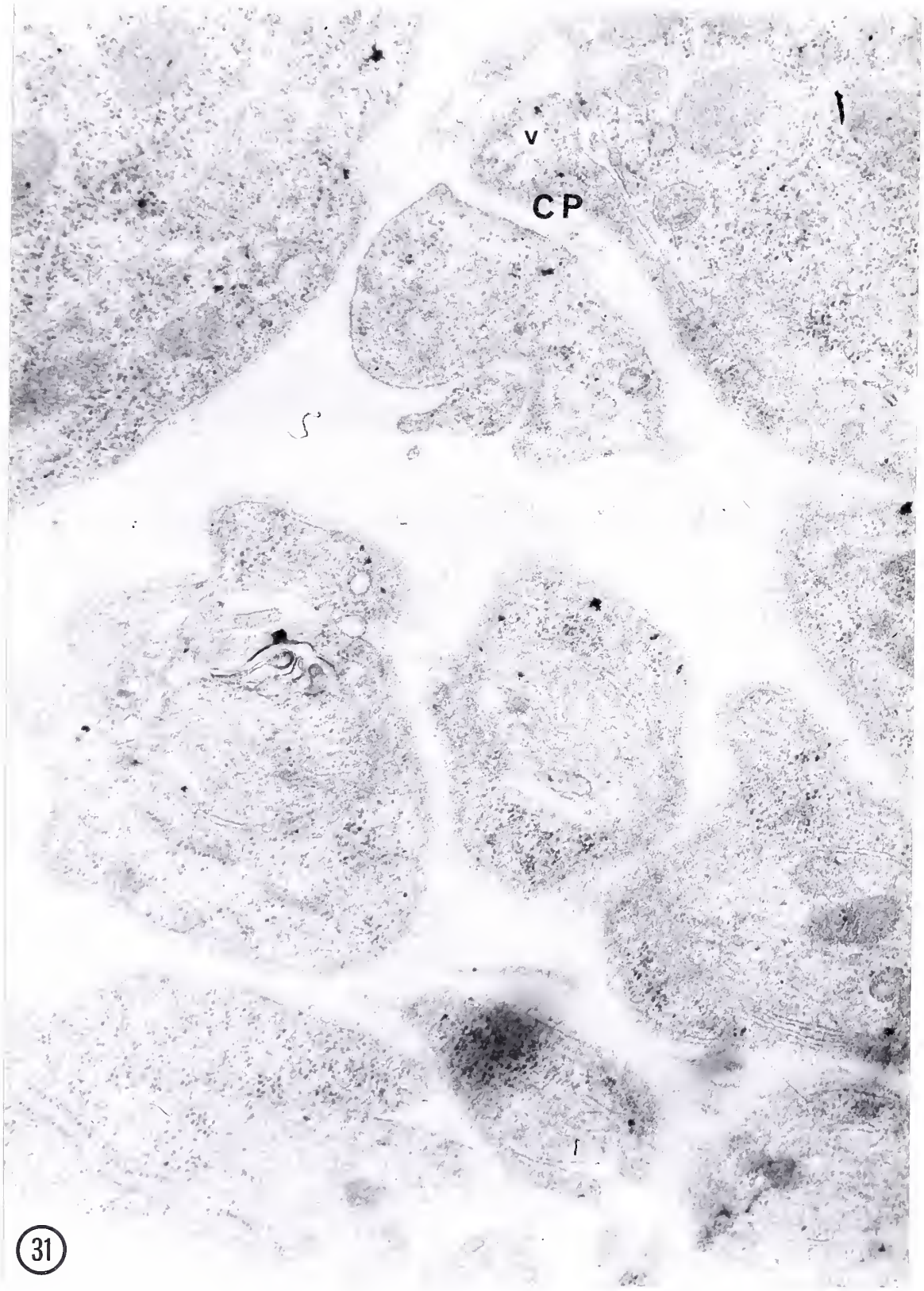

30

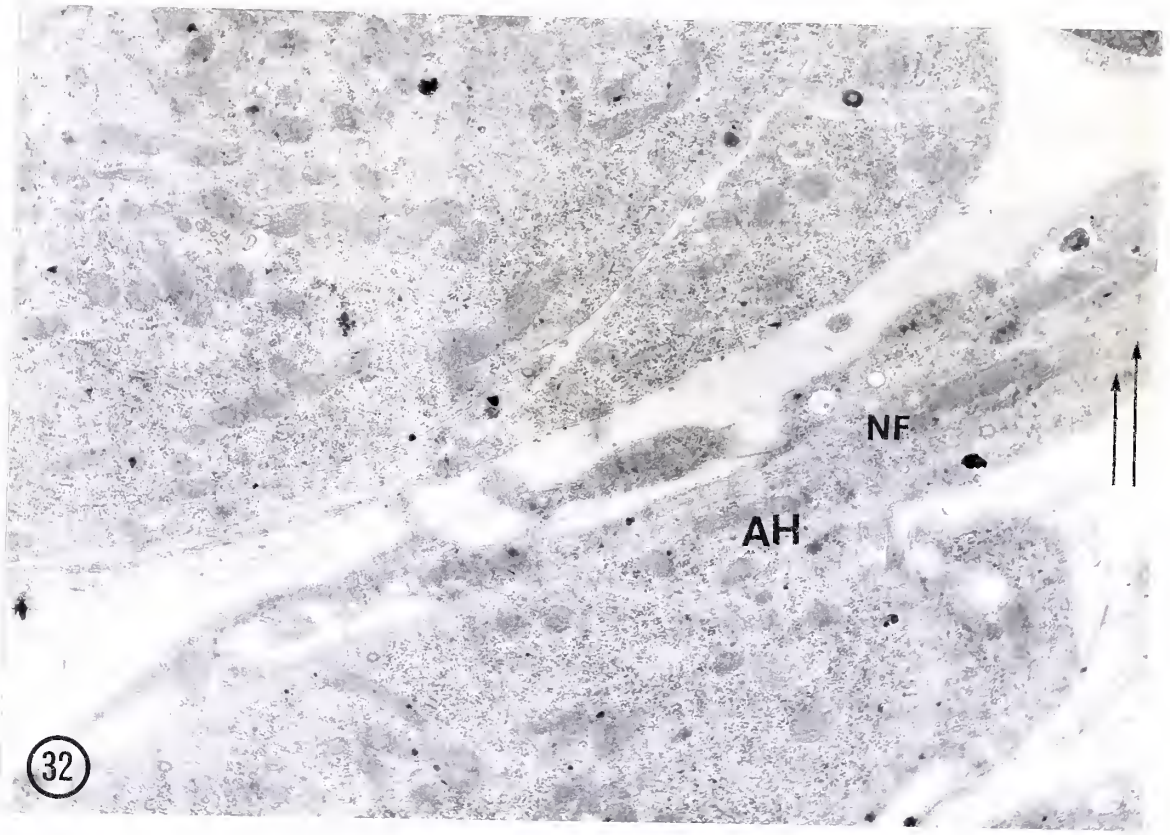
Figure 31. Electron micrograph of a five day mouse spiral ganglion. A characteristic "clubbed" process (CP) is apparent at the top of the photograph. Of note are the vacuoles (v) present in the satellite cell cytoplasm. X18,000.



31

Figure 32. Electron micrograph of a five day mouse spiral ganglion, showing an axon hillock (AH) region. The nerve fiber portion (NF) is ensheathed by two layers of cytoplasm, as denoted by the arrows. X8000.

Figure 33. Phase contrast micrograph of a seven day mouse spiral ganglion, showing well myelinated fibers cut longitudinally. X1800.



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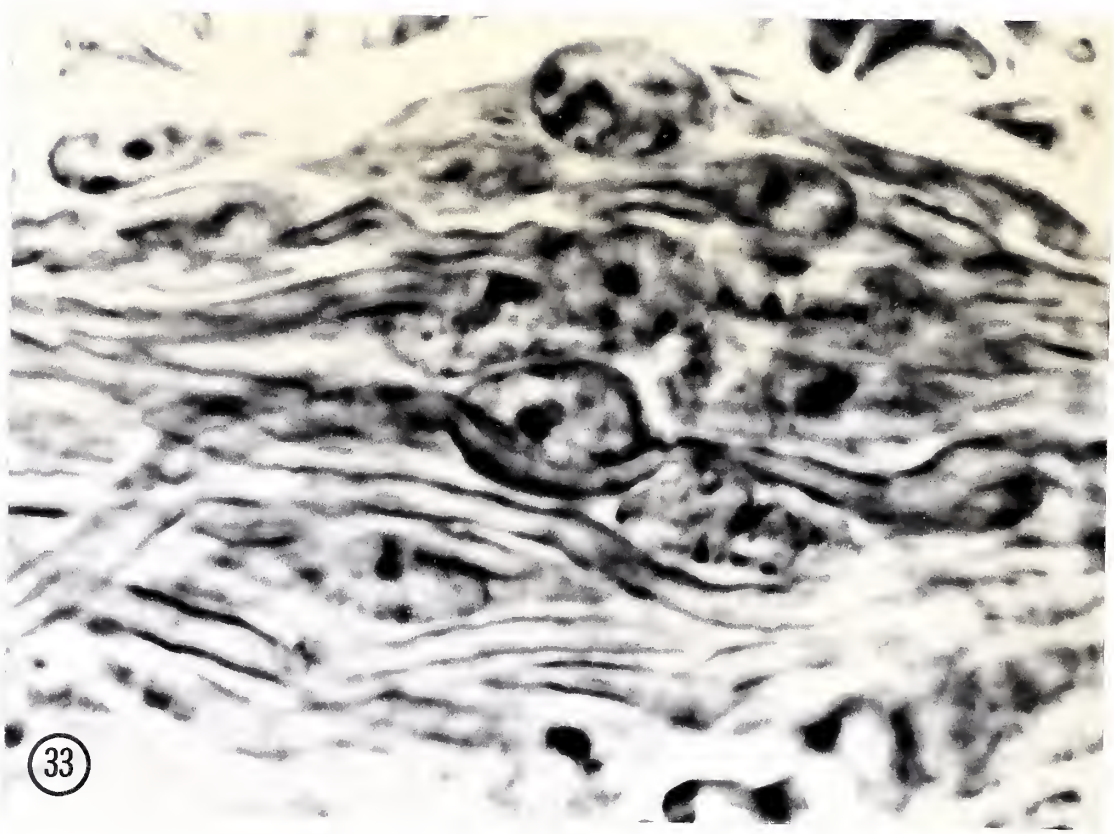
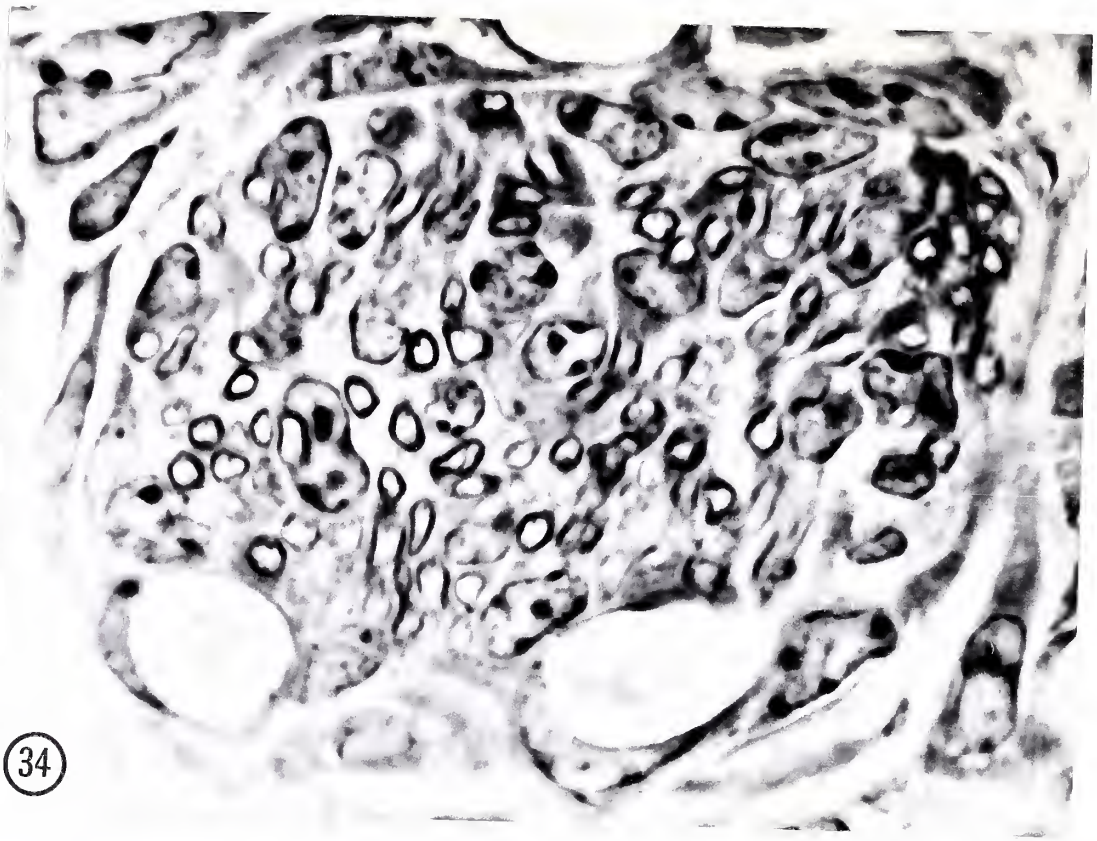


33

Figure 34. Phase contrast micrograph of a seven day mouse osseous spiral lamina, sectioned at right angles to the long axis of the nerve fibers. Note the varying thickness of the myelin sheaths. X1500.

Figure 35 Electron micrograph of a seven day mouse spiral ganglion, showing the region of an axon hillock (AH). An arrow points to the junction of the perikaryal satellite cell and the perineural Schwann cell. X35,000.



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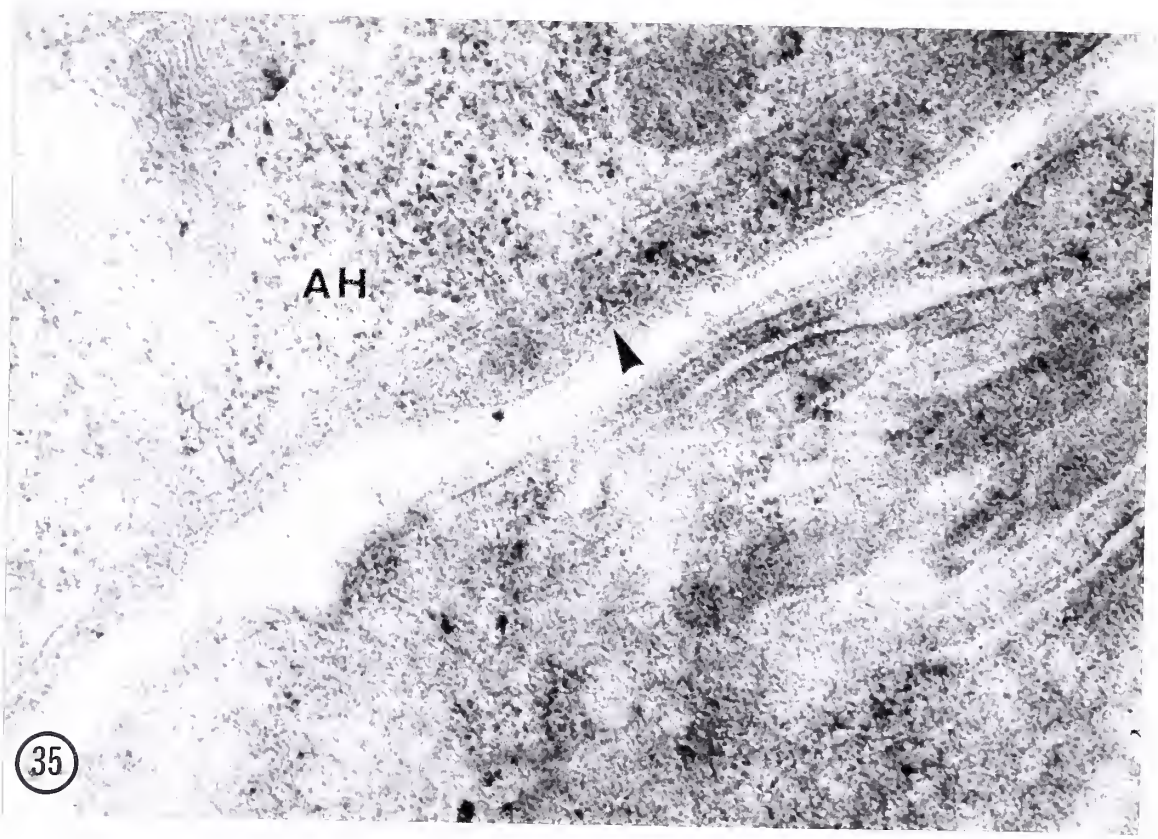
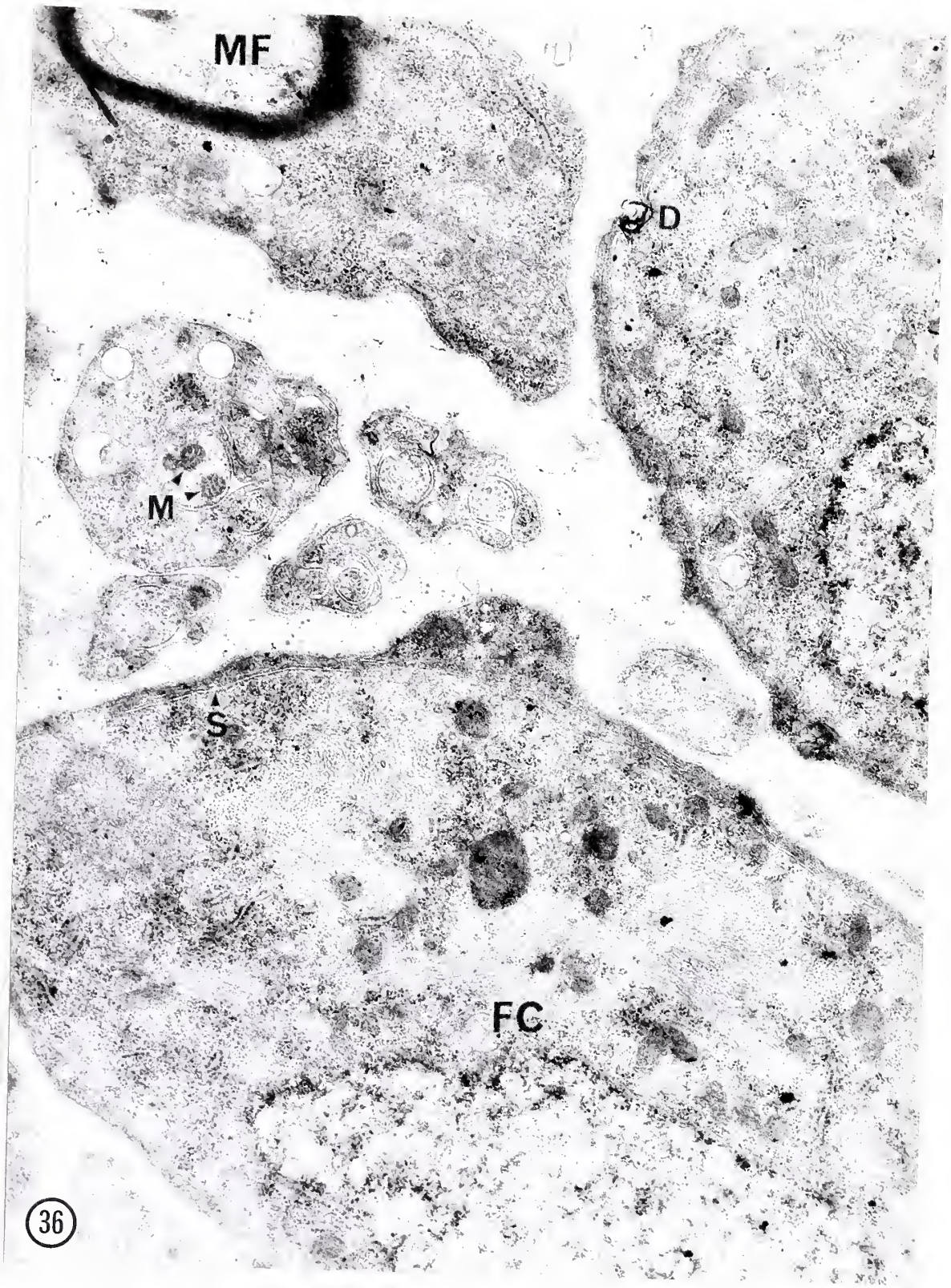


35

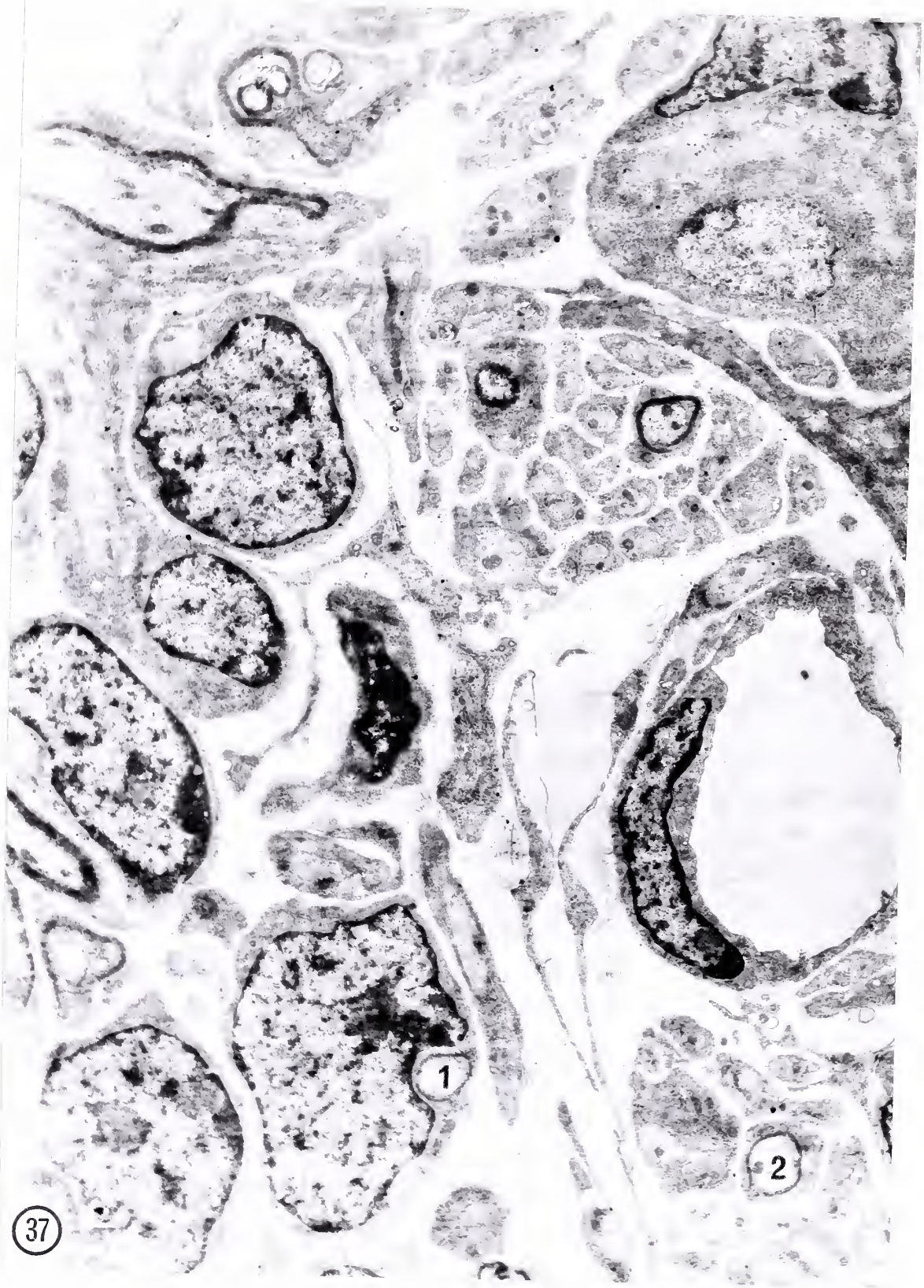
Figure 36. Electron micrograph of a seven day mouse spiral ganglion. A "filamented" ganglion cell (FC) is shown in the lower portion of the photograph with fine longitudinally oriented filaments in the cytoplasm. A subsurface cistern is located at (S) in that cell. In the adjacent cell, a rather bizarre structure, perhaps representing fusion of membranes is noted at (D).

Nerve fiber mitochondria appear with multiple transverse cristae (M) and differ from those seen in figure 21 at the newborn stage. In the upper left corner, a myelinated nerve fiber is shown (MF). X7000.



36

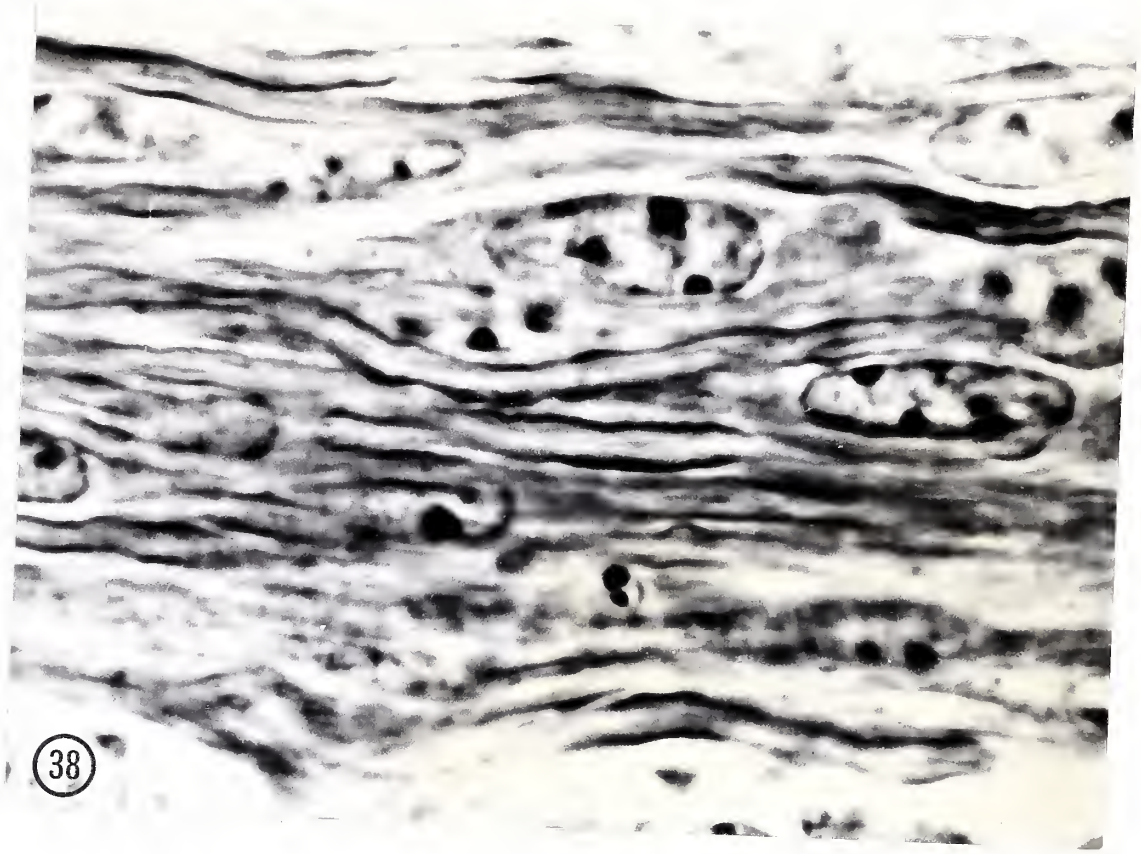
Figure 37. Electron micrograph of a seven day mouse spiral ganglion. At (1) and (2) are two lightly myelinated axons. The majority of the nerve fiber remain unmyelinated. X3000.



37

Figure 38. Phase contrast micrograph of a nine day mouse osseous spiral lamina showing a preponderance of myelinated nerve fibers. X 1800.

Figure 39. Phase contrast micrograph of a nine day mouse spiral ganglion. It is interesting that no change in the bipolar ganglion cell sheath is noted. X6600.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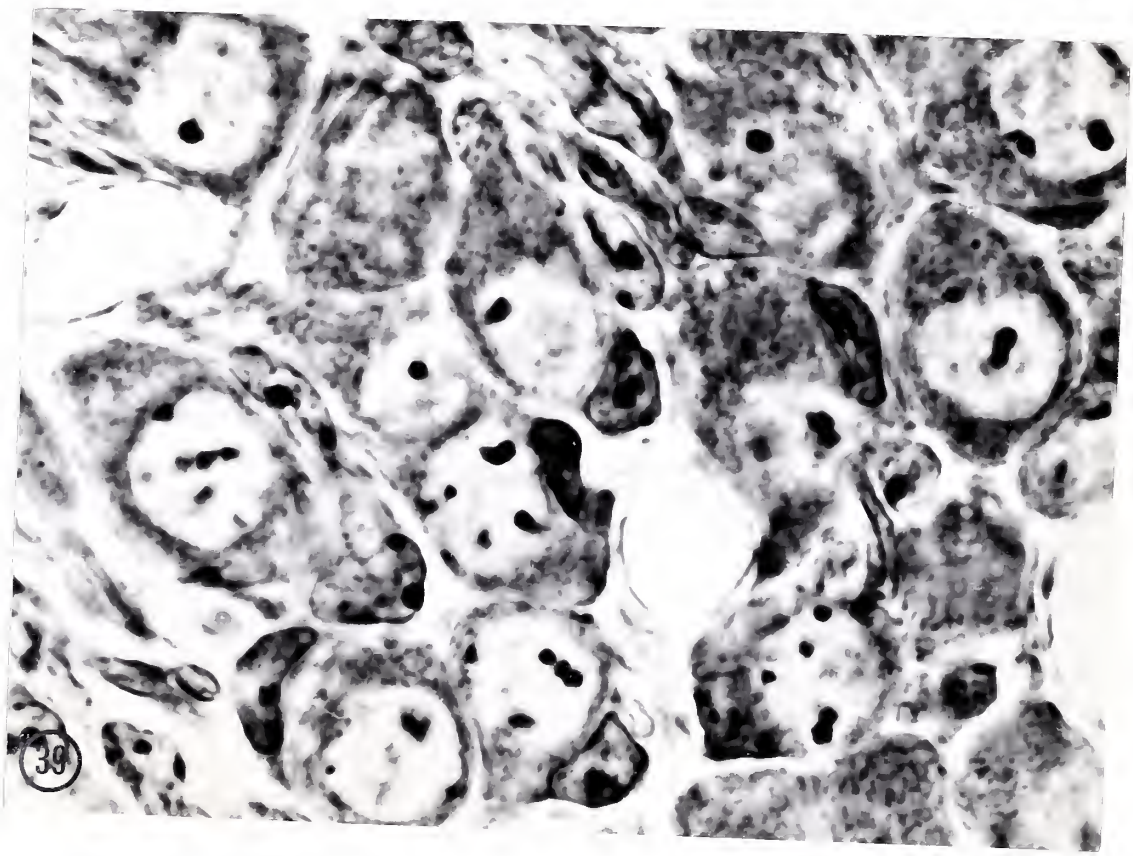
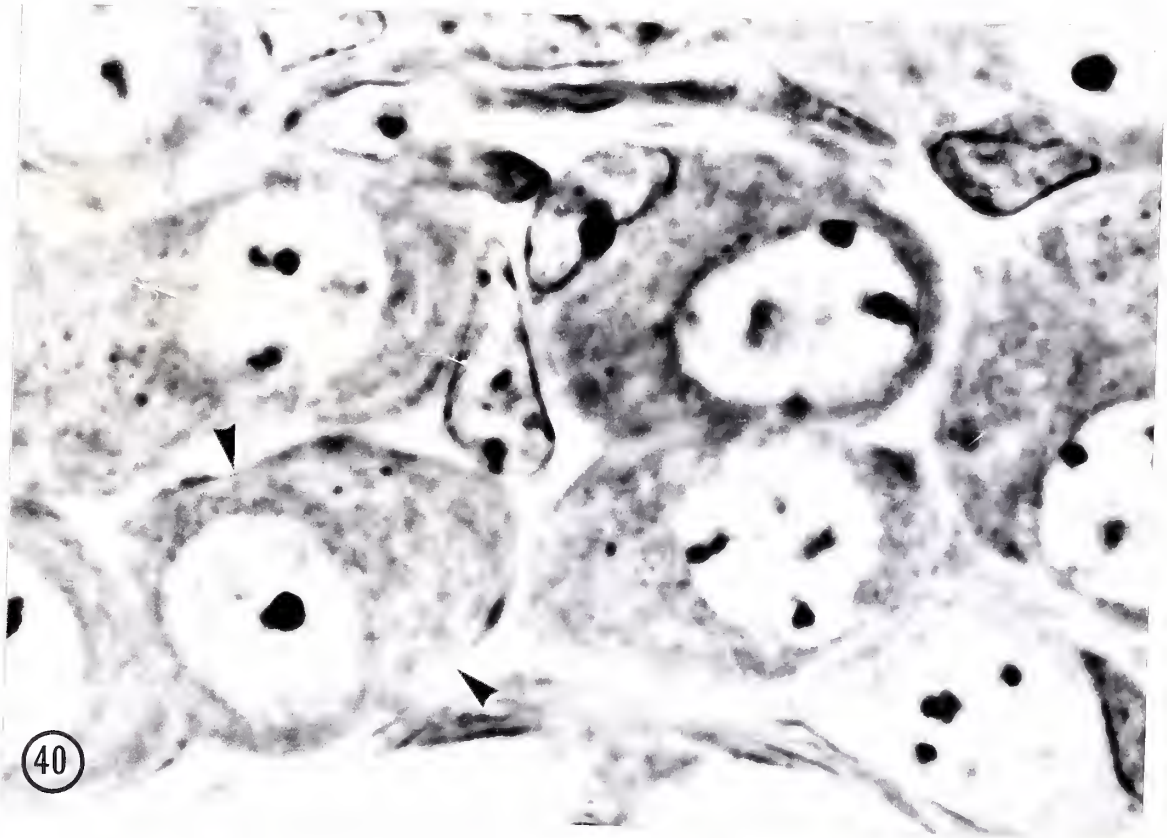

39

Figure 40. Phase contrast micrograph of an eleven day mouse spiral ganglion. Arrows point to slightly denser segments of satellite cell cytoplasm than seen previously. X1300.

Figure 41. Electron micrograph of an eleven day mouse spiral ganglion. Four layers of satellite cell cytoplasm are apparent between the two parallel lines. Two well myelinated nerve fibers are shown. X15,000.



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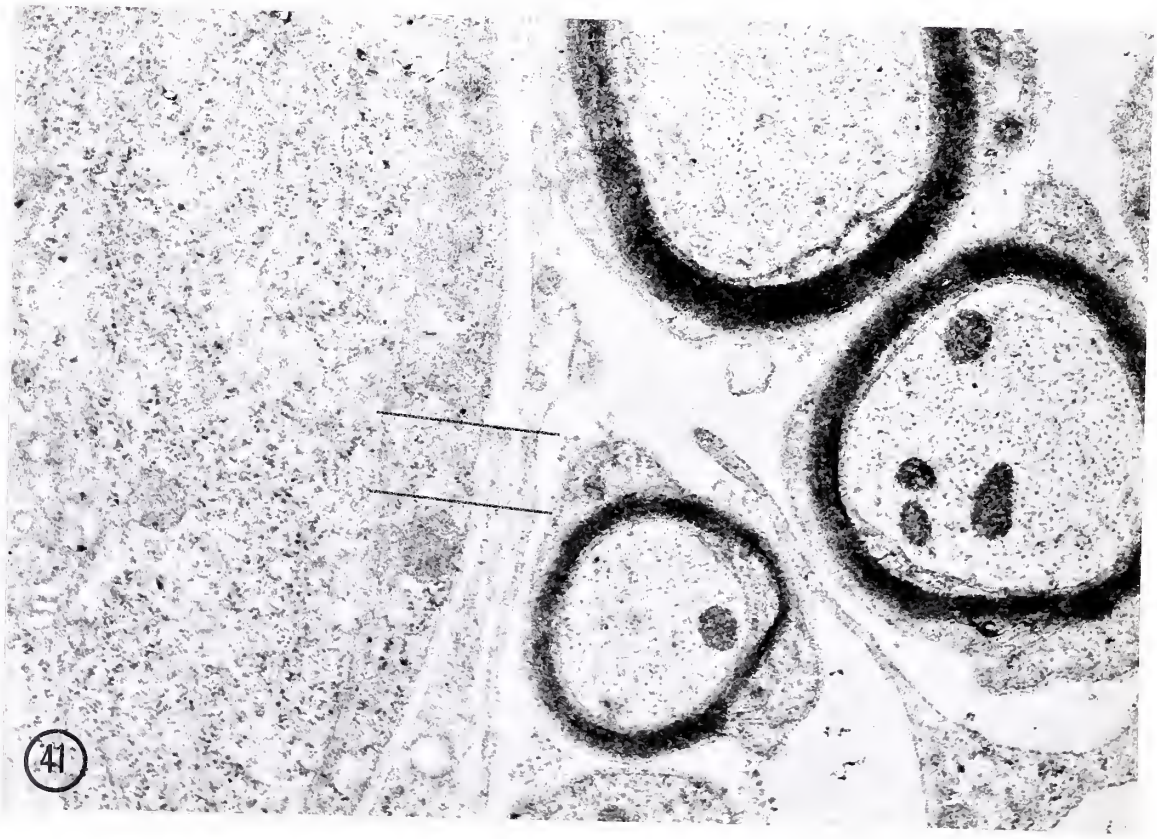
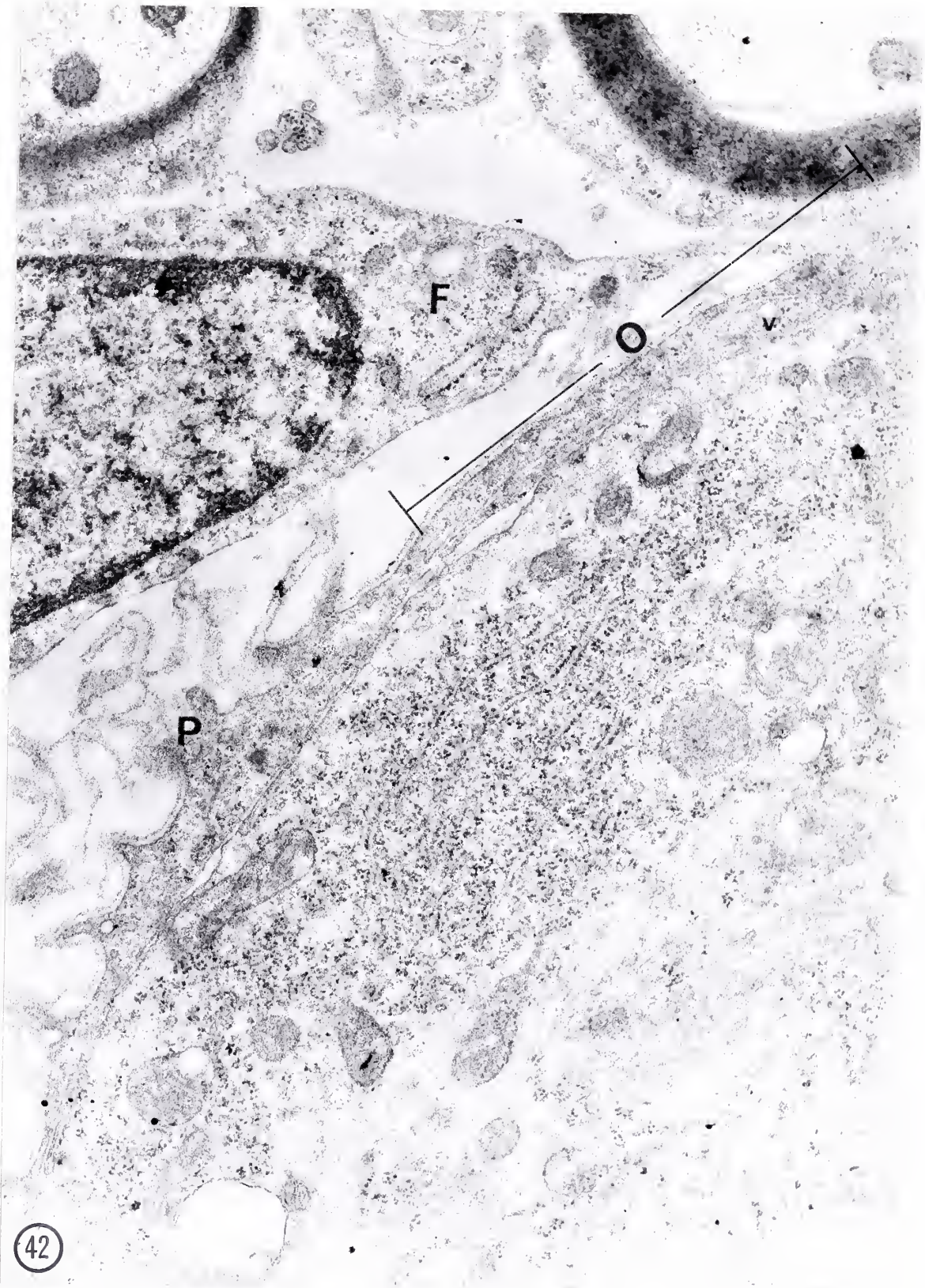


41

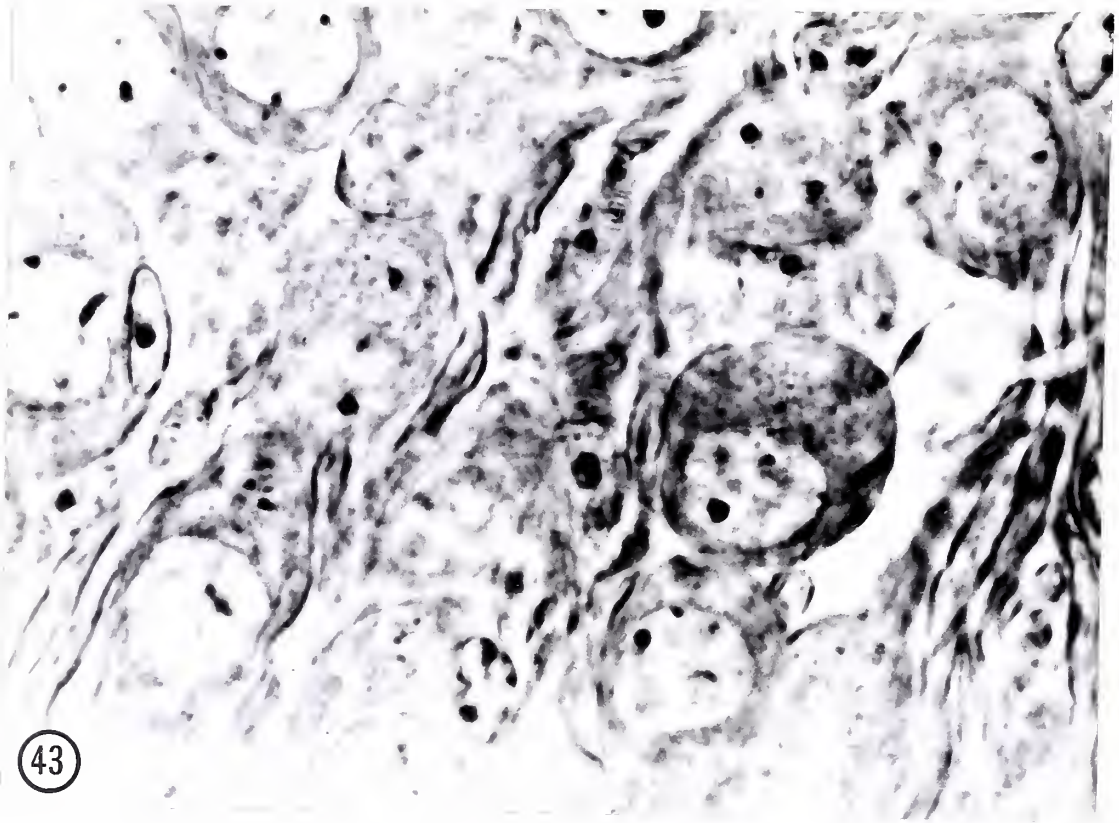
Figure 42. Electron micrograph of an eleven day mouse spiral ganglion. Note the area of overlap in satellite cell cytoplasm as denoted by (O) and the vacuole (v) present in the "clubbed" process. The pseudopodial configuration (P) is nicely demonstrated. A fibroblast (F), easily identified by its lack of basement membrane, is present. Interestingly, collagen fibriles are present in the periphery of that cell. X15,000.



42

Figure 43. Phase contrast micrograph of a twenty-two day mouse spiral ganglion. Of note are the dense sheaths encircling each neuron. X750.

Figure 44. Electron micrograph of a twenty-two day mouse spiral ganglion showing well defined satellite cell lamellations (PK) and the myelin sheath of a nerve fiber (m). X50,000.



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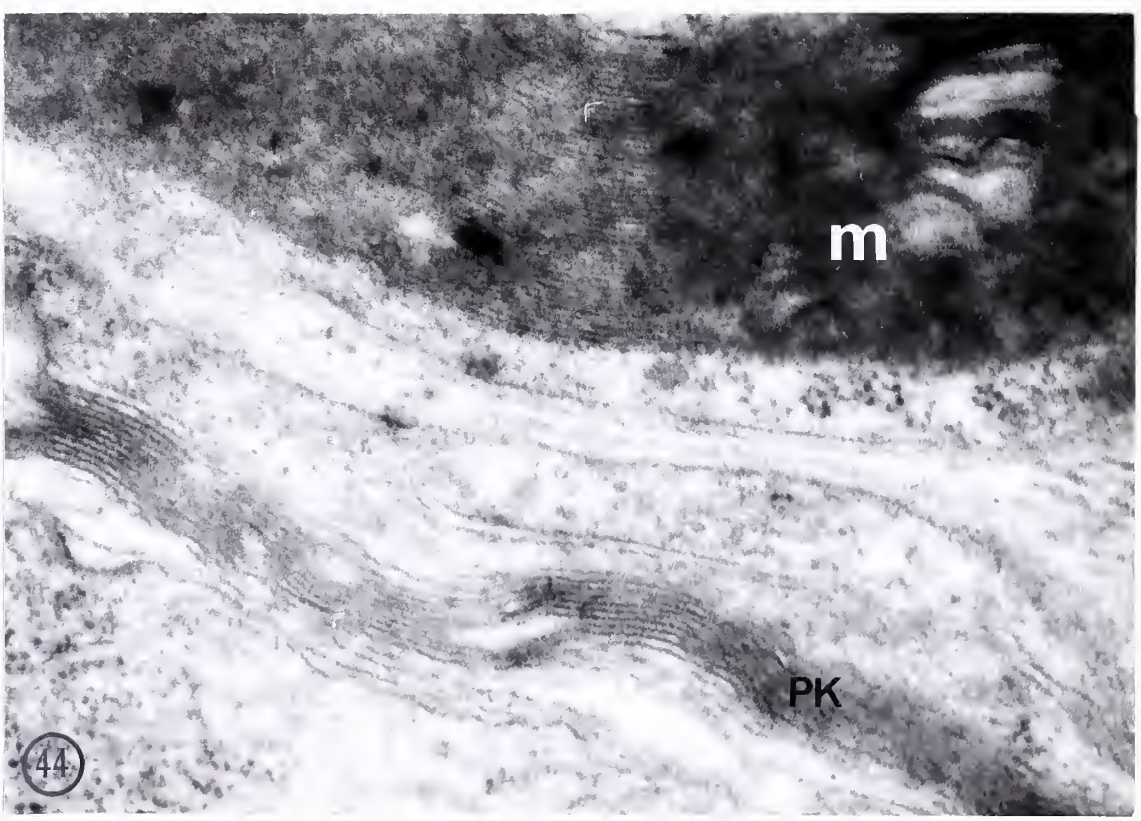


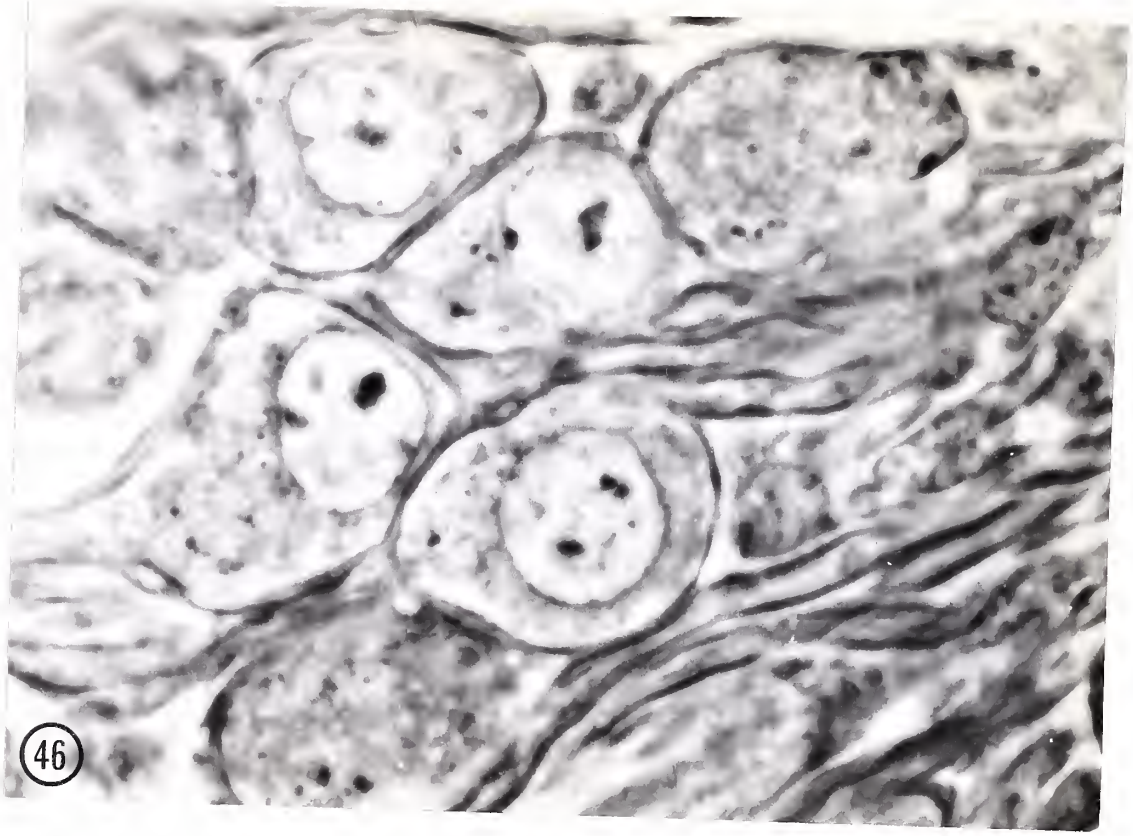
44

Figure 45. Electron micrograph of a twenty-two day mouse spiral ganglion showing multiple myelinated nerve fibers. Cytoplasmic bridges connecting different sheaths are denoted by arrows. At (C) can be seen a connection between a perikaryal sheath and cytoplasm surrounding a well myelinated nerve fiber. X10,000.

C
45

Figure 46. Phase contrast micrograph of an adult spiral ganglion. In comparison to the sheaths seen in figure 43, there is slightly more thickening. X1000.

Figure 47. Electron micrograph of an adult mouse spiral ganglion. X20,000.



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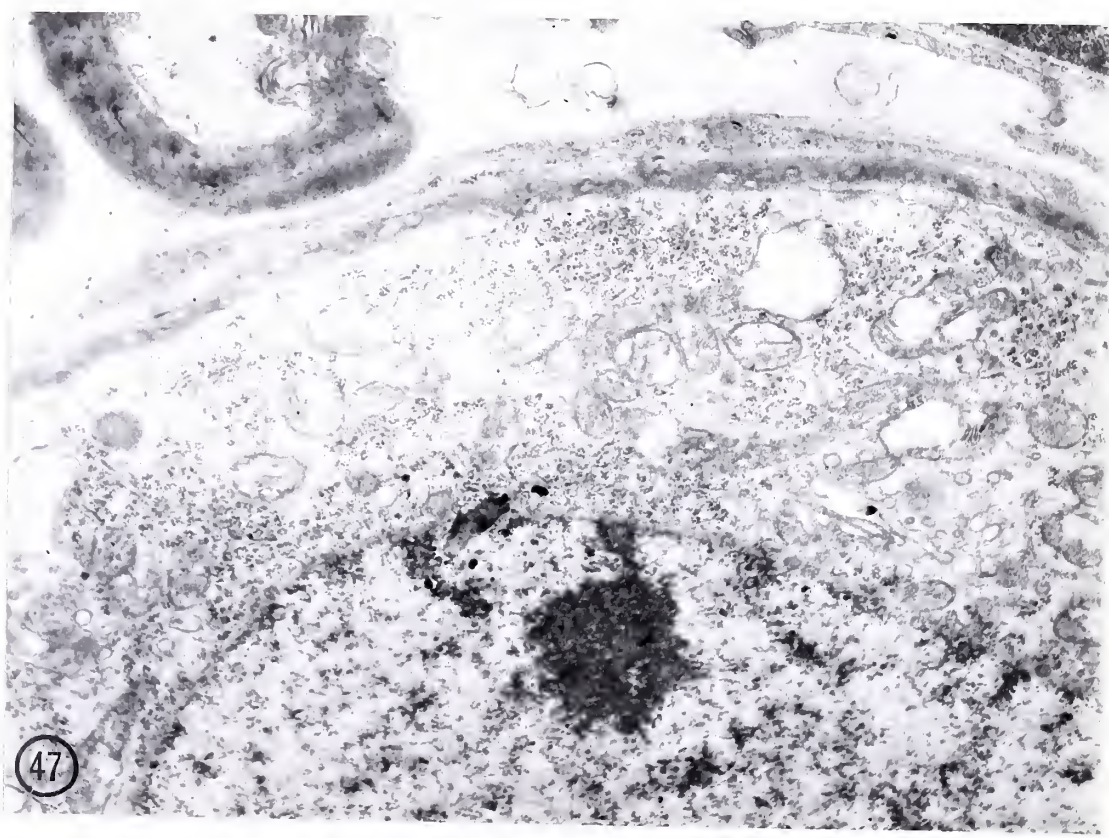
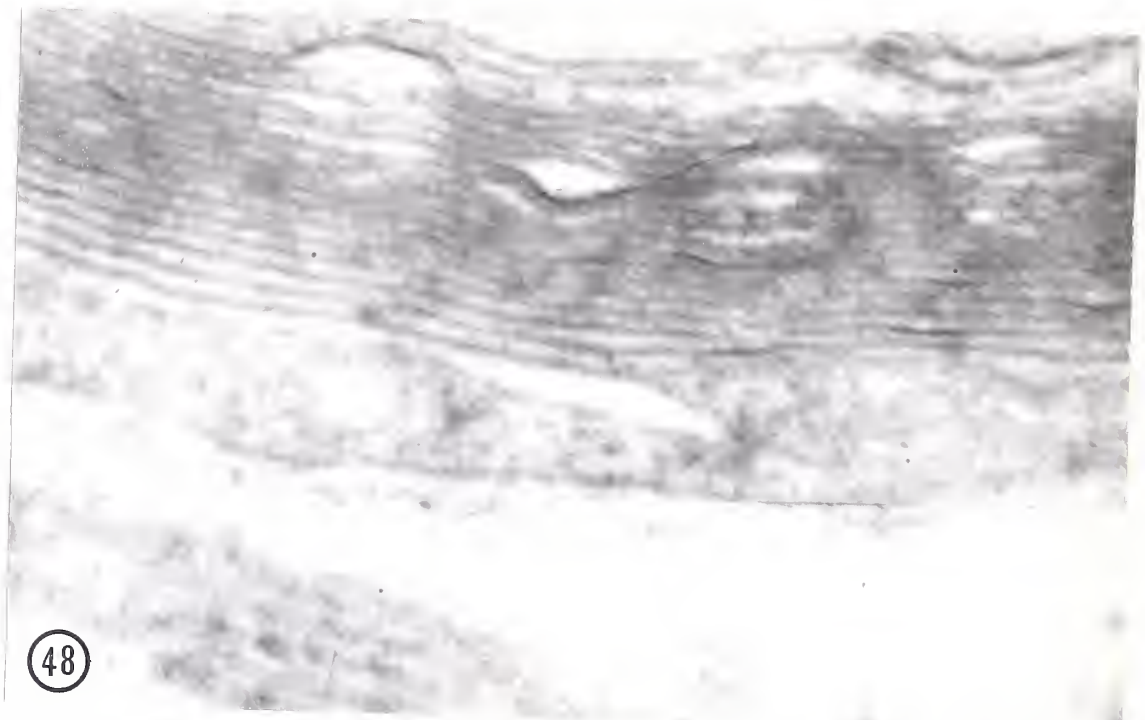


47

Figure 48. A high magnification electron micrograph of an adult perikaryal sheath showing multiple dense layers. X100,000.

Figure 49. Electron micrograph of an adult mouse spiral ganglion, showing a "T" formation comparable to that seen in figure 16. A node of Ranvier (NR) is seen cut obliquely. X15,000.



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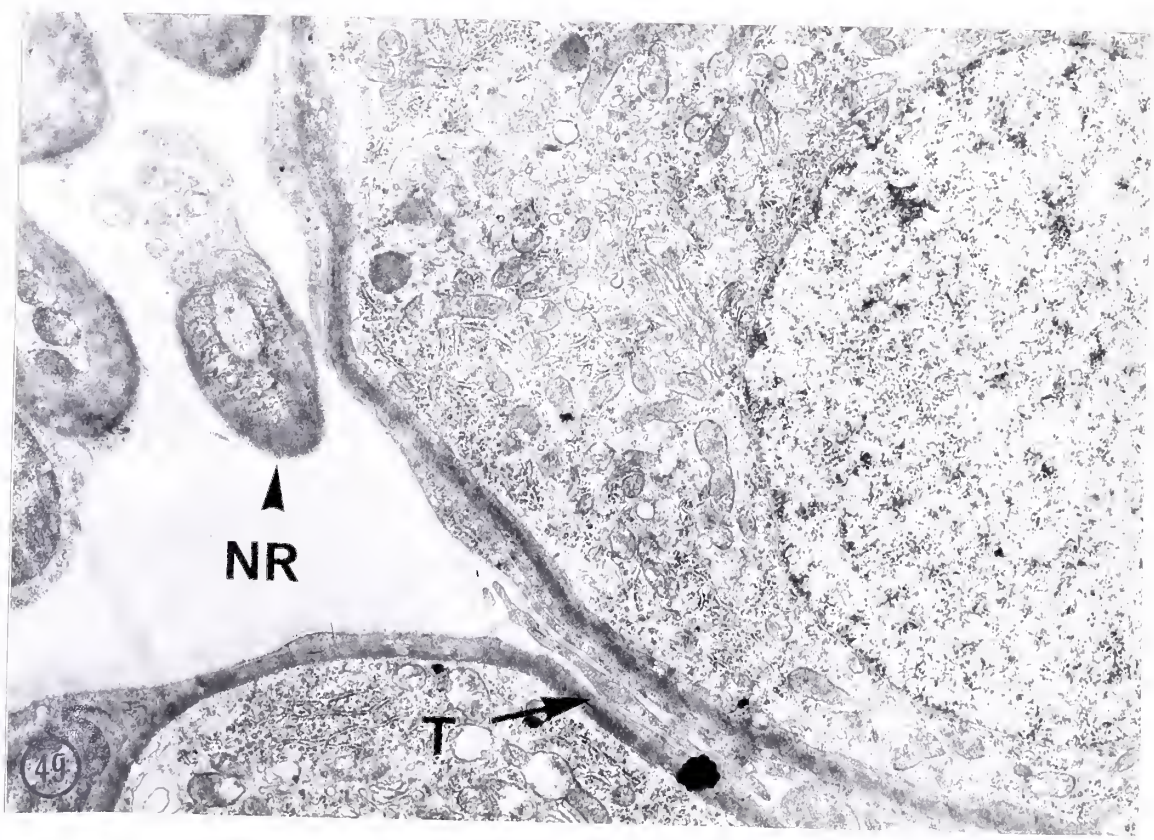
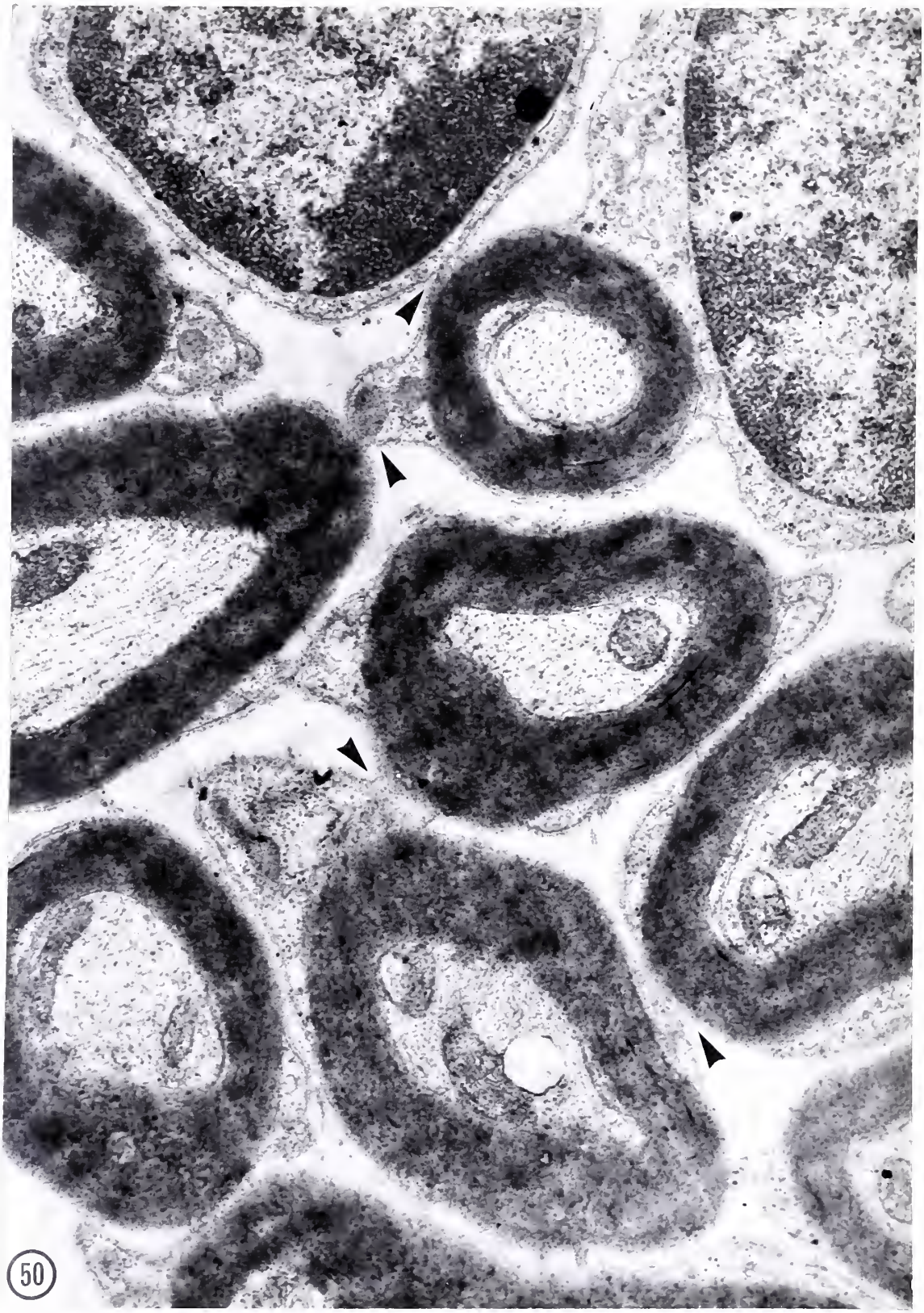


49

Figure 50. Electron micrograph of an adult mouse spiral ganglion. Arrows point out possible connections between Schwann cell cytoplasm enveloping different nerve fibers. X18,000.



50

Figure 51. Electron micrograph of an adult mouse spiral ganglion. Arrows point out possible cytoplasmic bridges. At (u) is an unmyelinated nerve fiber. X9000.

u

Figure 52. A modification from Cajal's drawing of a myelinated nerve fiber (1909). (A) The inner layer of Schwann cell cytoplasm. (B) The Schwann cell nucleus. (C) Schwann cell cytoplasm. (D) Sheath of Mauthner or axolemma. (E) Schmitt-Lantermann incisure. (F) Nerve fiber.

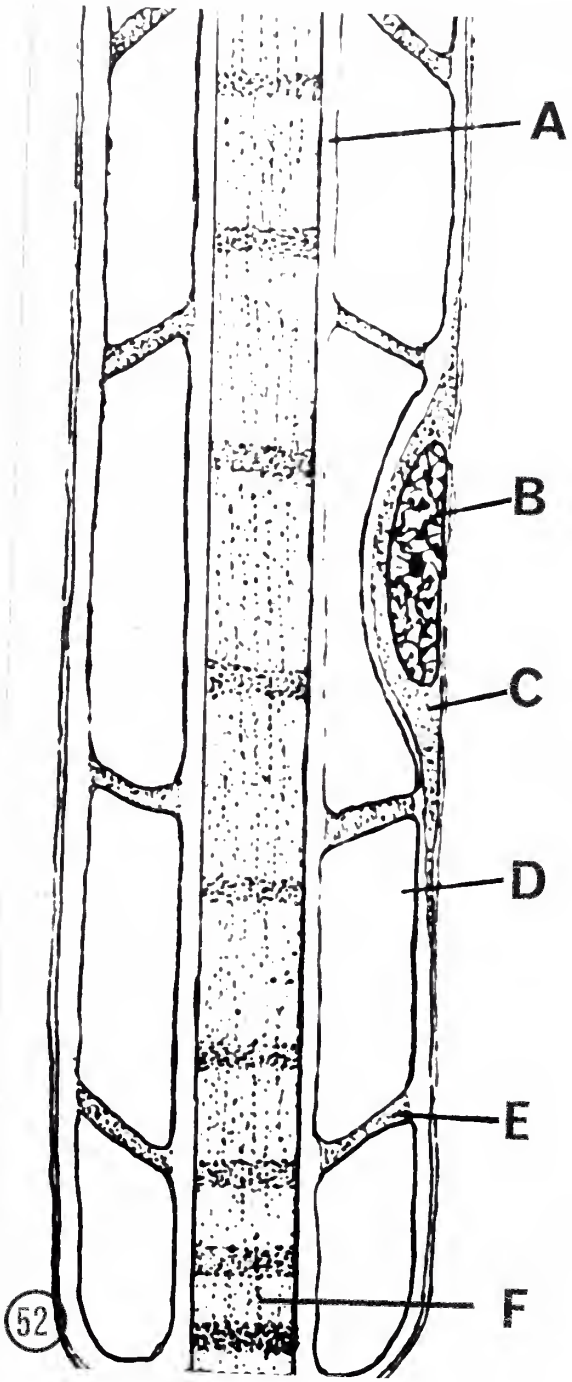
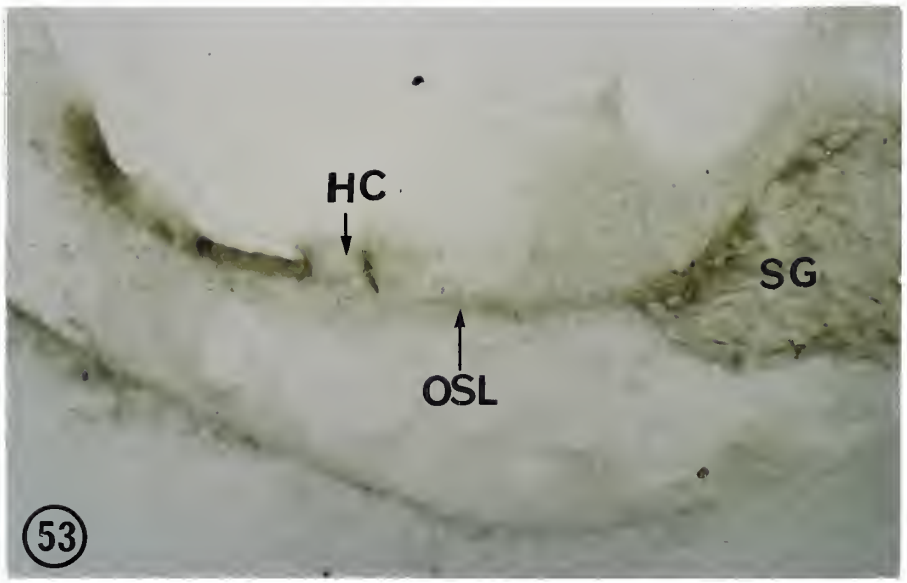
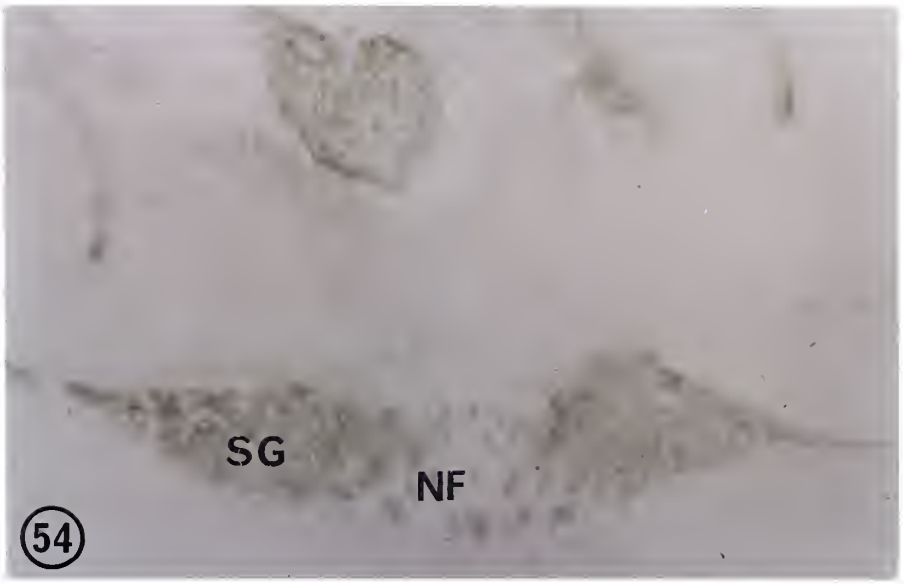

A
B
C
D
E
F
52

Figure 53. Light micrograph of a newborn organ of Corti and spiral ganglion. Note the moderate ACHE activity in the region of the hair cells (HC) and the more intense staining lateral to those cells. The spiral ganglion (SG) and osseous spiral lamina (OSL) show ACHE activity. The section from which this and the following photographs are taken are from specimens perfused with formalin, sectioned in the cryostat, and stained for cholinesterase using the Gomori method. X500.

Figure 54. Light micrograph of a newborn cochlea sectioned along the axis of the modiolus. ACHE activity is identified by darker staining in the region of the eighth nerve fibers (NF) and in the spiral ganglion (SG). X300.

Figure 55. Light micrograph of a three day mouse cochlea and brainstem sectioned parallel to the long axis of the modiolus and stained for ACHE activity. Note the marked staining in the entire eighth nerve (N) from its point of egress from the brainstem to the hair cell region. X85.


HC
SG
OSL
53

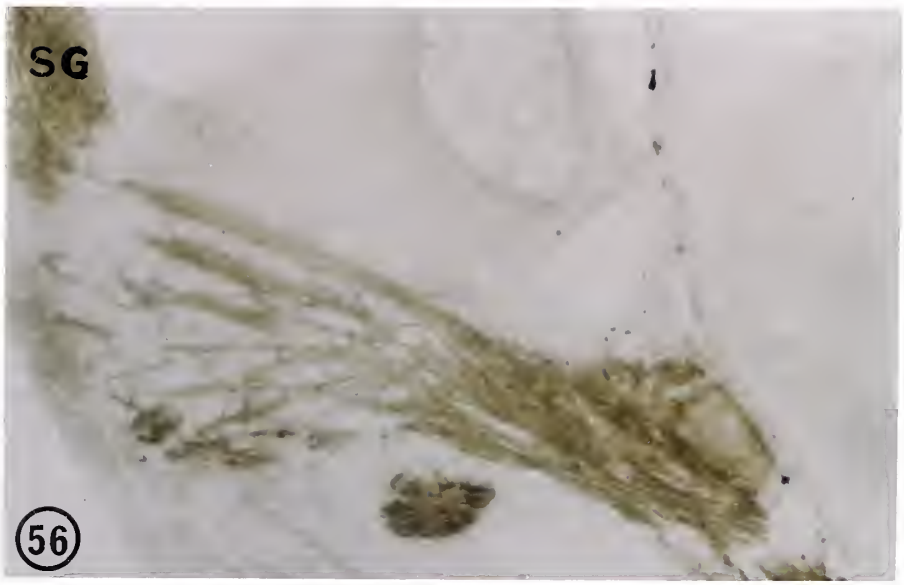

SG
NF
54

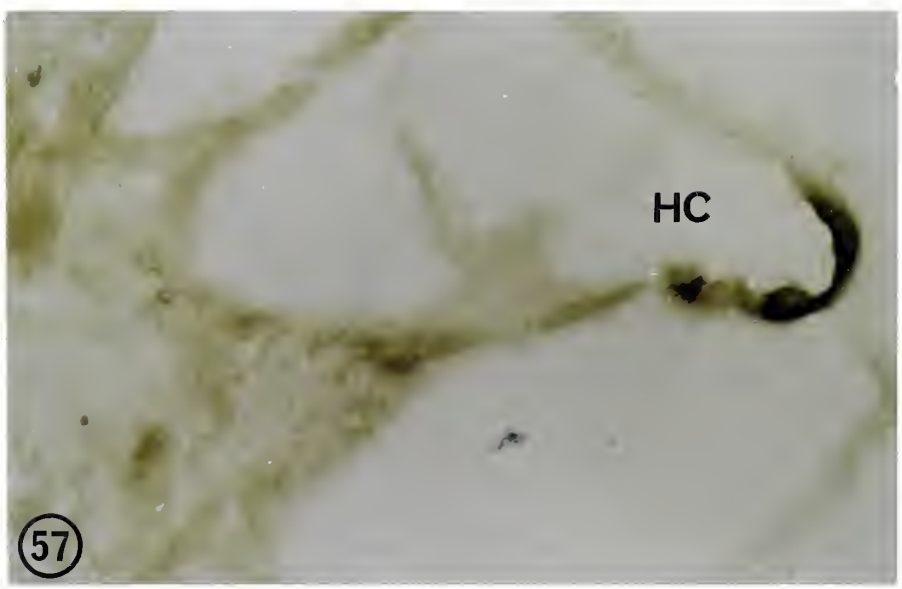
N
55

Figure 56. Light micrograph of a five day mouse cochlea sectioned along the axis of the modiolus. ACHE activity is present throughout the eighth nerve from its point of egress from the brainstem. The spiral ganglion (SG) stains strongly. It is quite interesting that the brainstem in the five day mouse (at the right in the photograph) shows almost no cholinesterase activity whereas the activity in the adult brainstem is quite striking (Fig. 60). X300.

Figure 57. Light micrograph of a five day mouse organ of Corti stained for ACHE activity. More intense activity is noted about the hair cell region (HC). X250.

Figure 58. Light micrograph of a seven day mouse cochlea sectioned at right angles to the long axis of the modiolus. The efferent spiral bundle (ESB) is noted by an arrow. The spiral ganglion is located immediately below the spiral bundle in the photograph. No ACHE is apparent in the cell bodies. The region of the osseous spiral lamina (OSL) is immediately above the efferent spiral bundle extending to the region of the organ of Corti (OC). Hair cells are readily recognized by their hexagonal shape and regularity (HC). X250.


SG
56


HC
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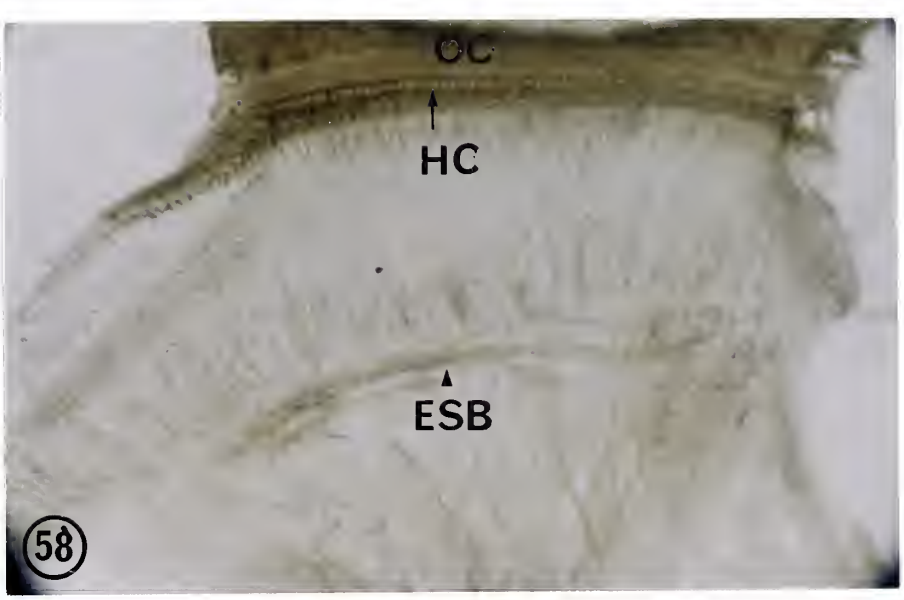
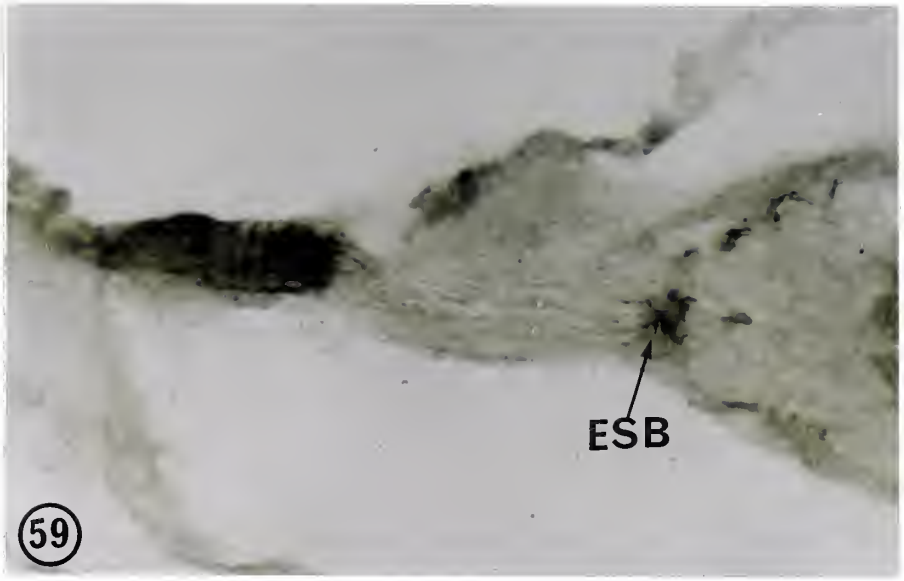

OC
HC
ESB
58

Figure 59. Light micrograph of a seven day mouse organ of Corti and spiral ganglion. Note the intense ACHE activity in the region of the efferent spiral bundle (ESB) and in the organ of Corti,with the relative lack of activity elsewhere. X300.

Figure 60. Adult mouse brainstem at the region of the genu (G) of the facial nerve. The superior olivary nucleus (SON) and efferent fibers (EF) are stained intensely for AChe activity. The efferent fibers are seen to join the afferent component of the eighth nerve shortly before those fibers course to the cochlear nuclei. X40.

Figure 61. Adult mouse brainstem sectioned slightly distal to the specimen in figure 60. Efferent fibers are seen to be present at the point of exit of the eighth nerve from the brainstem. X40.


ESB
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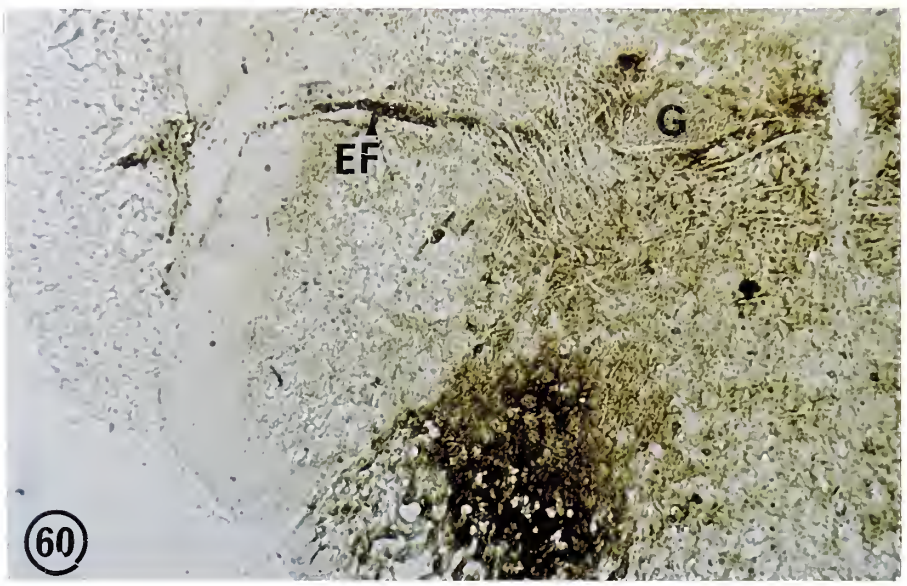

EF
G
60

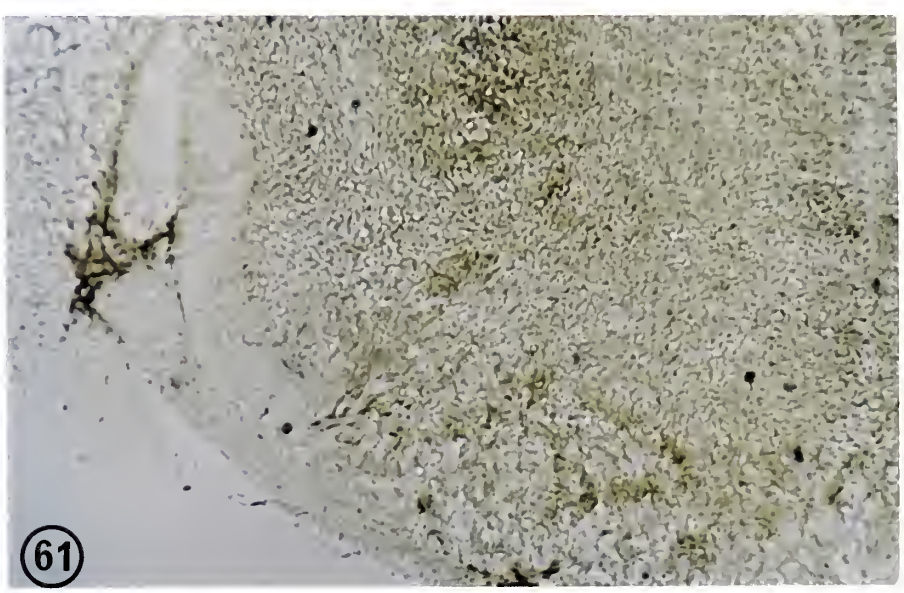
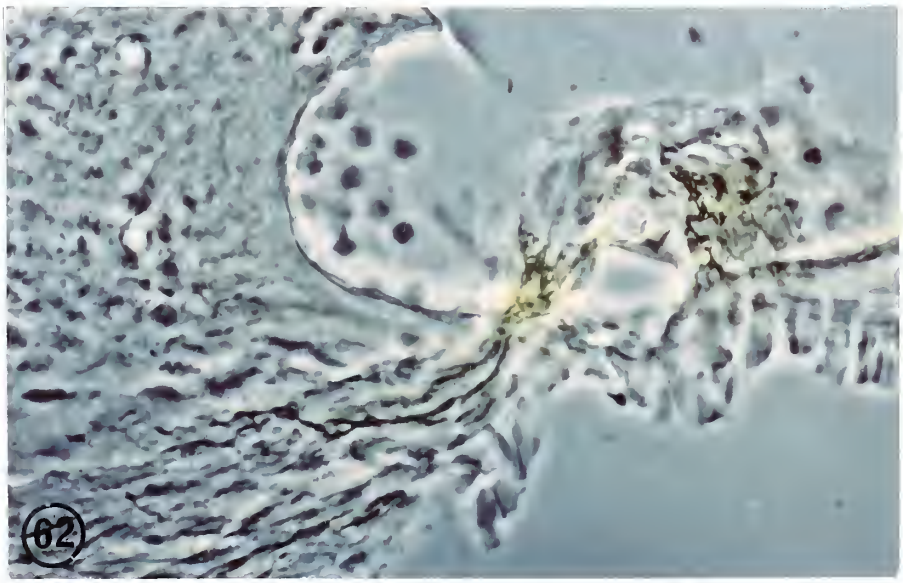

61

Figure 62. Phase contrast micrograph of an adult organ of Corti stained for ACHE activity. Such activity is present rather distinctly at the base of the hair cells. X1200.

Figure 63. Newborn mouse cochlea stained for ACHE activity but including pre-incubation and incubation with BW c251. No activity is seen in the region of the nerve fibers, spiral ganglion (SG), or organ of Corti (O). Very slight staining is present in the cells lateral to the hair cells. X300.

Figure 64. Newborn mouse cochlea stained for ACHE activity. Note the activity in the region of the nerve fibers (NF), spiral ganglion (SG), organ of Corti, and cells lateral to that organ. X280.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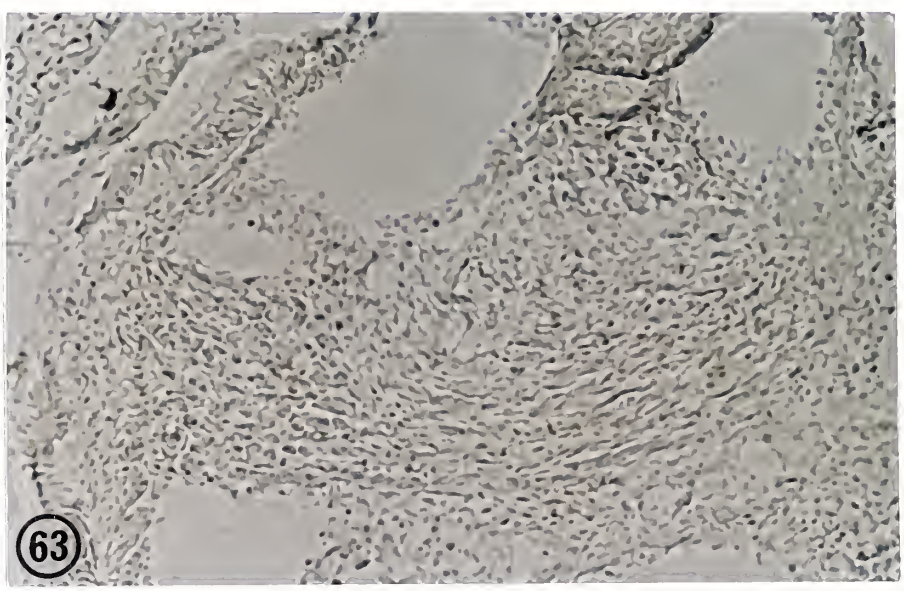
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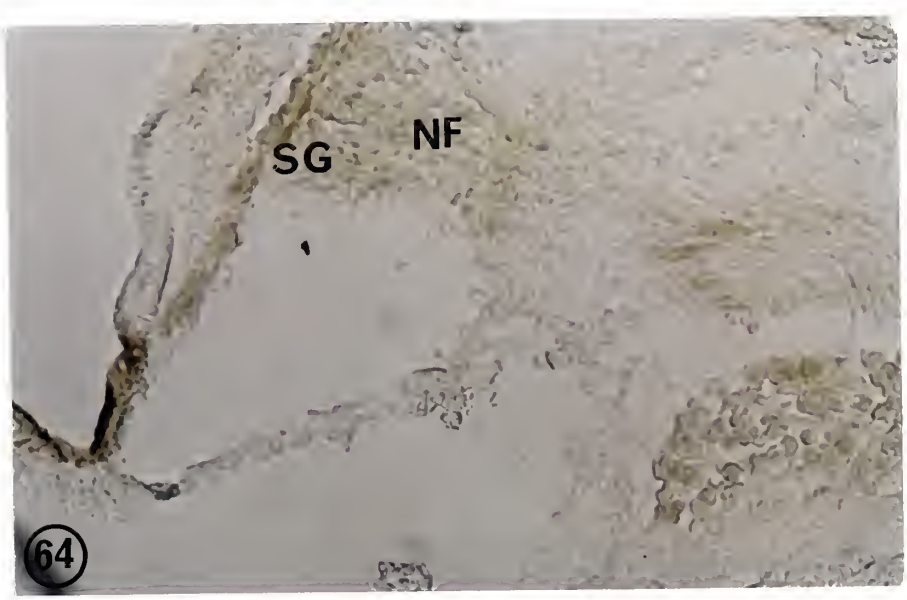
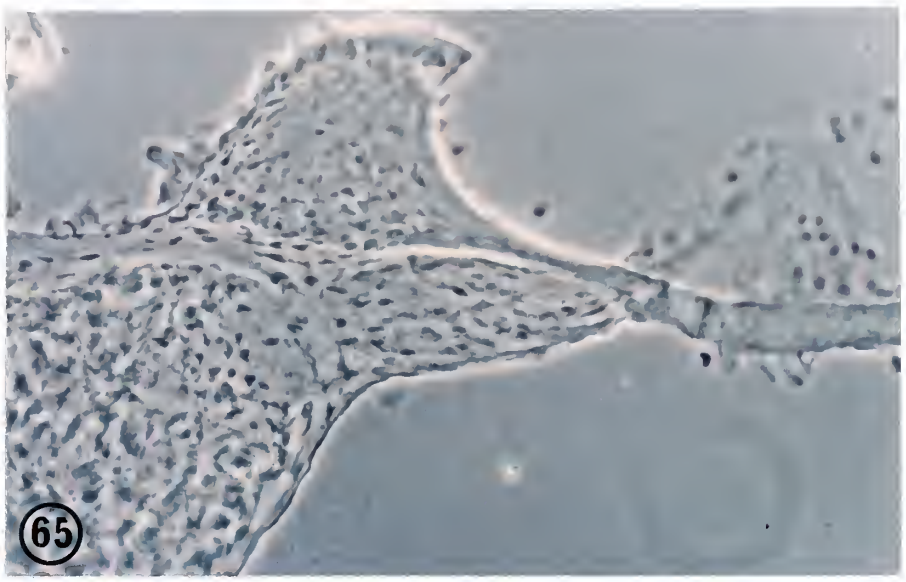

SG
NF
64

Figure 65. Adult mouse organ of Corti stained for ACHE but including pre-incubation and incubation with BW c251. No staining for ACHE is noted. X1100.

Figure 66. Light micrograph of the eighth nerve in the modiolus of a one day mouse cochlea. Note the absence of blue staining denoting phospholipid in the nerve fibers (NF). X1000.

Figure 67. Light micrograph of a cochlea in a one day mouse. Note the absence of phospholipid staining in the region of the spiral ganglion (SG). X1000.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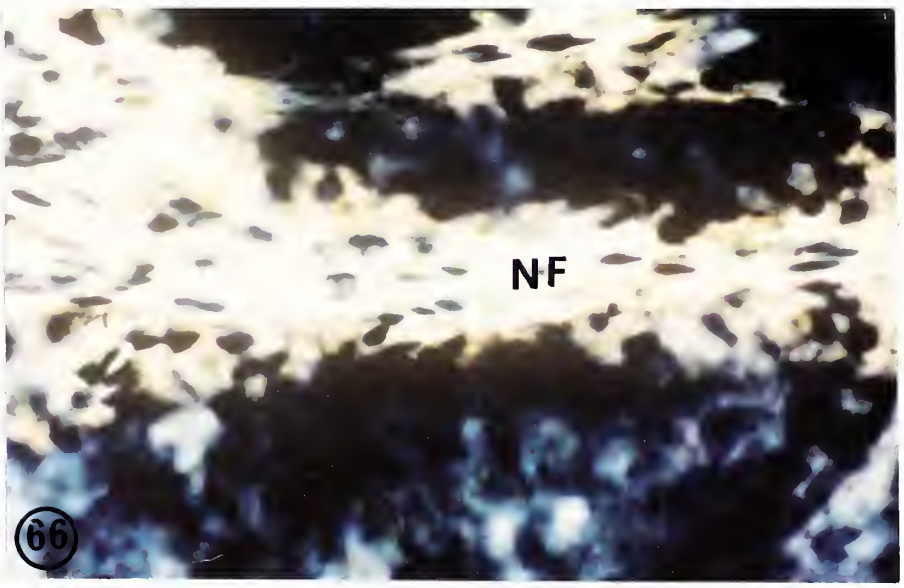
NF
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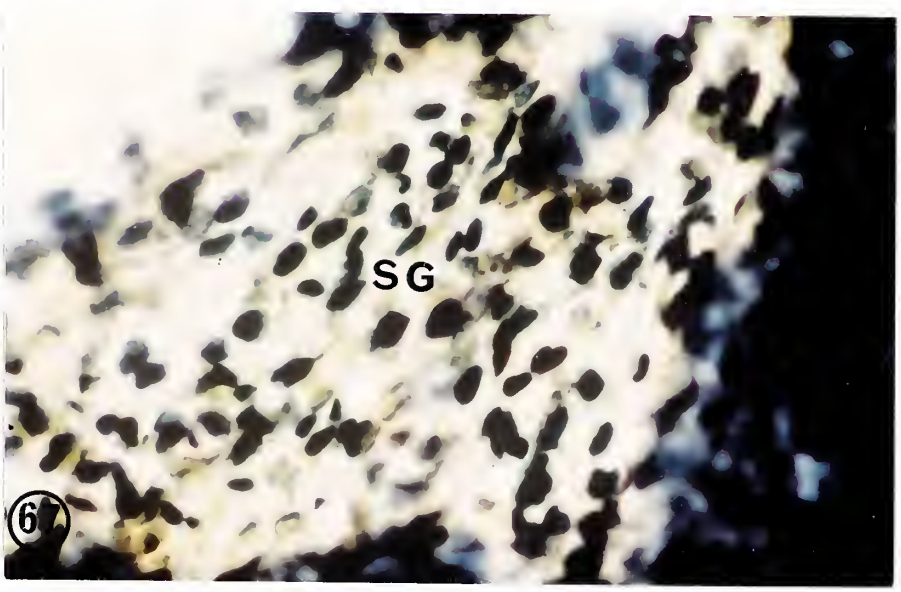
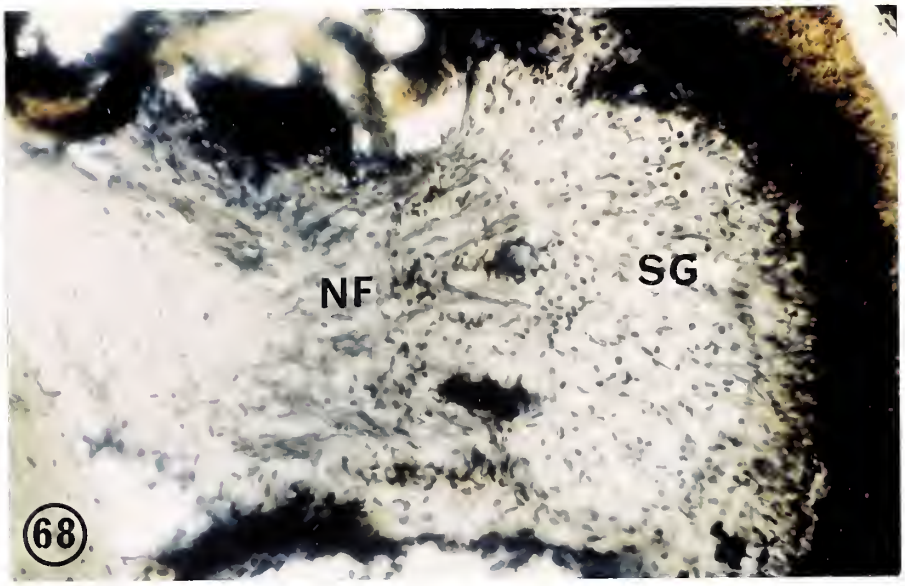
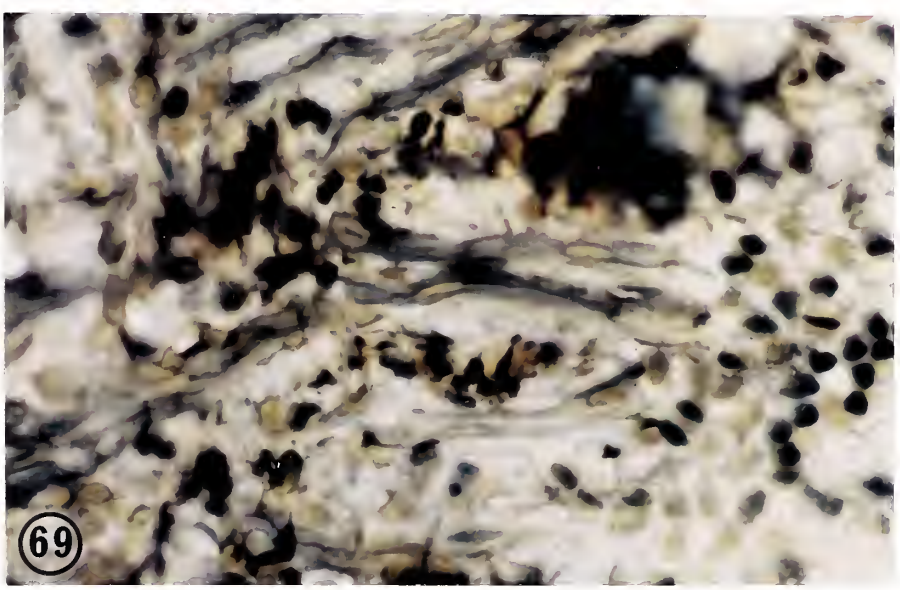

SG
67

Figure 68. Light micrograph of a five day mouse cochlea sectioned along the axis of the modiolus and stained for phospholipid. Note the faintly stained nerve fibers in the region marked NF. The spiral ganglion (SG) is located on the right in the photograph. X300.

Figure 69. Light micrograph of a five day mouse cochlea showing nerve fibers outlined denoting accumulation of phospholipids or what might be termed "chemical" myelination. X1200.

Figure 70. Light micrograph of a five day mouse spiral ganglion showing very faintly outlined ganglion cells. X1200.


NF
SG
68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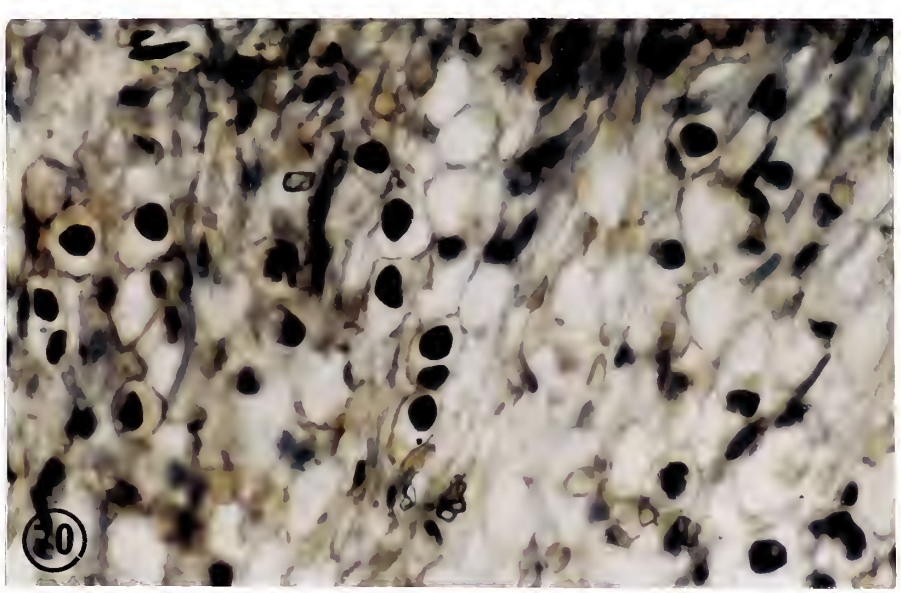
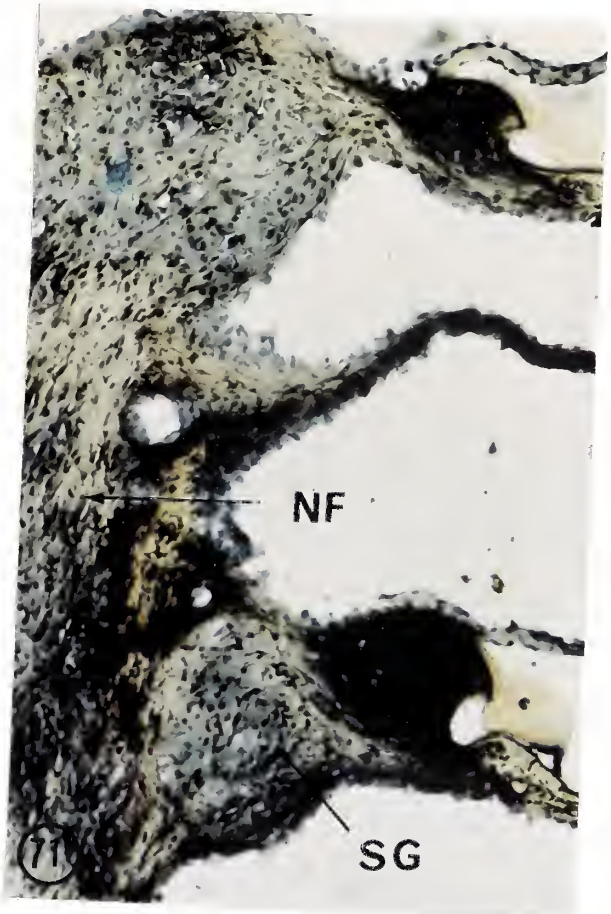

70

Figure 71. Light micrograph of a seven day cochlea stained for phospholipid. Staining is more intense in the nerve fiber region (NF) in the mid-basal area. Slight coloration is noted in the spiral ganglion (SG), especially in the lower spiral ganglion region. X300.

Figure 72. Light micrograph of a nine day mouse cochlea sectioned along the long axis of the modiolus and stained for phospholipid. Nerve fibers are stained intensely especially in the regions of the spiral bundle (SB) and in the fibers leading to the spiral ganglion (SG). Only moderate staining is present tin the latter area at the apex. X300.


NF
SG
71

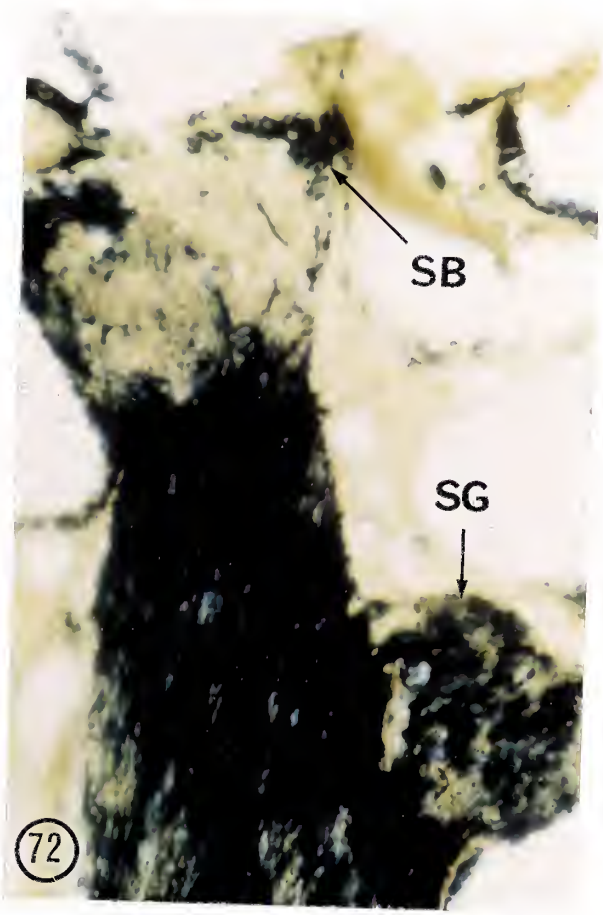

SB
SG
72

Figure 73. Light micrograph of an adult cochlea showing spiral ganglion and organ of Corti. The section is stained for phospholipid. Intense coloration is present in the nerve fiber region. X250.

Figure 74. Light micrograph of an adult spiral ganglion stained for phospholipid. A band of blue coloration can be seen outlining each cell, signifying myelination. X400.

73

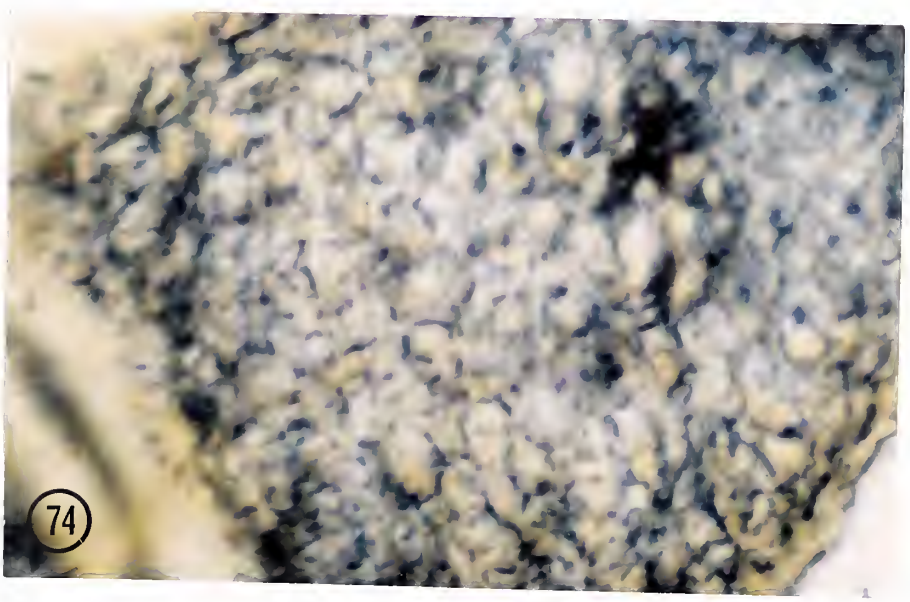

74

Figure 75. Graphic representation of the fine structure of cell membranes and the process by which fusing of Schwann cell unit membranes produces the myelin sheath. (Reproduced from Ham (1961), permission from the author and publisher applied for.)

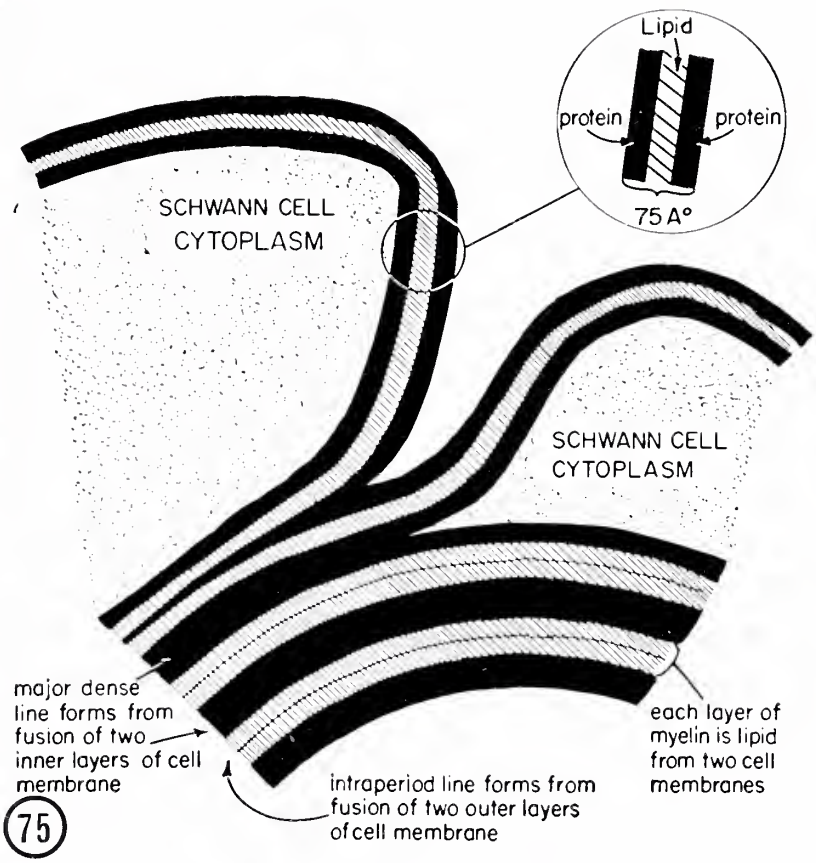
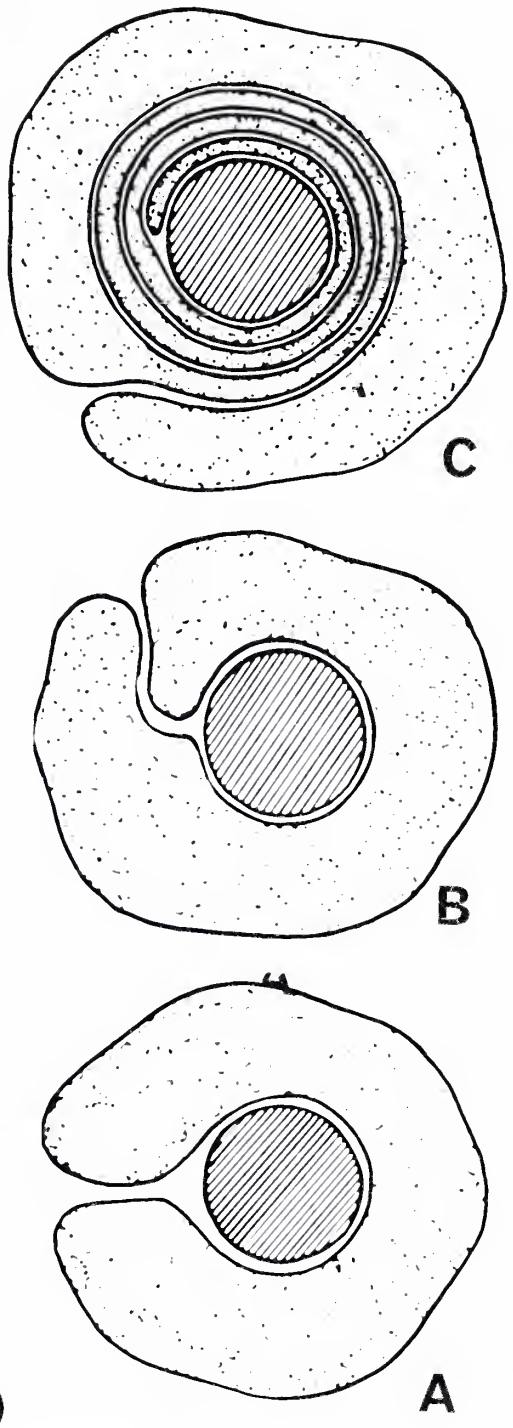

Lipid
protein
protein
75 A°
SCHWANN CELL
CYTOPLASM
SCHWANN CELL
CYTOPLASM
major dense
line forms from
fusion of two
inner layers of cell
membrane
intraperiod line forms from
fusion of two outer layers
of cell membrane
each layer of
myelin is lipid
from two cell
membranes
75

Figure 76. Diagram representing the early stages of the formation of the myelin sheath. (Reproduced from Ham (1961), permission from the author and publisher applied for.)


C
B
A

BIBLIOGRAPHY

Alford, B.R. and R.J. Ruben. Physiological, behavioral, and anatomical correlates of the development of hearing in the mouse. Ann. Otol. 72, p. 237, 1963.

Baker, J.R. Histochemical recognition of lipids. Quart. J. Micr. Sci. 87, p. 441, 1946.

Balogh, K., Jr. and Y. Nomura. A technique for the demonstration of acetylcholinesterase activity in the inner ear after decalcification with EDTA. J. Histochem. and Cytochem. 12, p. 931, 1964.

Bethe, A. Neue versuche über die regeneration der nervenfasern. Pflugers Arch. f. d. ges. Physiol. 116. p. 385, 1907. Cited in: Nageothe, J. Sheaths of the peripheral nerves. In Cytology and Cellular Pathology of the Nervous System. ed. Penfield, W. Paul B. Hoeber. New York. vol. I. p. 189, 1933.

Boettcher, A. Weitere beiträge qur anatomie der schnecke. Arch. Path. Anat. 17, p.243, 1859. Cited in Rossi, G., 1961.

Borghesan, E. Alterazioni cocleari e vestibalari consecutive a lesione sperimentale del nervo acustico. Atti clinica O.R.L. Univ. di Roma. 28, p. 107, 1930. Cited in Rossi, G., 1961.

Bunge, M.B., R.P. Bunge, G.D. Pappas. Electron microscopic demonstration of connections between glia and myelin sheaths in the developing mammalian nervous system. J. Cell. Biol. 12, p. 448, 1962.

Bunge, R.R. and P.M. Glass. Some observations on myelin-glial relationships and on the etiology of the cerebrospianl fluid exchange lesion. Annals N.Y. Acad. Sci. 122, p. 15, 1965.

Causey, G. The Cell of Schwann. Livingstone Press, Edinburgh. 1960.

Churchill, J, H. Schuknecht, and R. Doran. Acetylcholinesterase activity in the cochlea. Laryngoscope. 66, p.1, 1956.

Davis, H. Some principles of sensory receptor action. Physiol. Rev. 41, p. 391, 1961.

Davis, H. Advances in the neurophysiology and neuroanatomy of the cochlea. J. Acoust. Soc. Amer. 34, p. 1377, 1962.

DeRobertis, E., H.M. Gerschenfeld, and F. Wald. Cellular mechanisms

of myelination in the CNS. J. Biophys. and Biochem. Cytol. 4, p. 651, 1958.

Desmedt, J.E. and P. Monaco. Mode of action of the efferent olivocochlear bundle on the inner ear. Nature. 192, P. 1293, 1961.

Desmedt, J.E. and V. Lagoutta. Function of the uncrossed efferent olivocochlear fibers in the cat. Nature. 200, p. 472, 1963.

Dixon, A.D. The ultrastructure of nerve fibers in the trigeminal ganglion of the rat. J. Ultrastruc. Res. 8, p. 107, 1963.

Engström, H. and F.S. Sjöstrand. Structure and innervation of the cochlear hair cells. Acta Oto -Laryng. 44, p. 490, 1954.

Engström, H. On the double innervation of sensory epithelia of the inner ear. Acta Oto-Laryng. 49, p. 109, 1958.

Engström, H. Electron micrographic studies of the receptor cells of the organ of Corti. In: Neural Mechanisms of the Auditory and Vestibular Systems. ed. Rasmussen, G.L. and W.F. Windle. C.C. Thomas Co., Springfield. 1960.

Erlanger, J. and H.S. Gasser. Electrical Signs of Nervous Activity. Univ. of Penn. Press, Phila. 1937.

Ernyei, S. and M.R. Young. Pulsatile and myelin-forming activities of Schwann cells in vitro. J. Physiol. (London). 183, p. 469, 1966.

Fernandez, C. The innervation of the cochlea. Laryngoscope. 61, p. 1152, 1951.

Fernandez-Moran, H. Electron microscope observations on the structure of the myelinated nerve fiber sheath. Exptl. Cell Res. 1, p. 143, 1950.

Fex, J. Augmentation of the cochlear microphonics by stimulation of efferent fibers to the cochlea. Acta Oto-Laryng. 50, p.540, 1959.

Fex, J. Auditory activity in centrifugal and centripetal cochlear fibres in the cat. Acta Physiol. Scand. 55, Suppl. 189, 1962.

Gacek, R.R., Y. Nomura, and K. Balogh. Acetylcholinesterase activity in the efferent fibers of the stato-acoustic nerve. Acta Oto-Laryng. 59, p. 542, 1965.

Galambos, R. Suppression of auditory nerve activity by stimulation of efferent fibers to the cochlea. J. Neurophysiol. 19, p. 424, 1956.

Gasser, H. Discussion on saltatory conduction hypothesis. Cold Springs Harbor Symposium. Quant. Biol. 17, p. 32, 1952.

Gerebtziff, M.A. Non-specific cholinesterase in myelin sheath of the peripheral fiber: Histochemical localization: Effects of stimulation and section. C.R. Soc. Biol. (Paris). 158, p. 1171, 1964.

Geren, B.B. Development of the fine structure of the myelin sheath in sciatic nerves of chick embryos. Proc. Natl. Acad. Sci. U.S. 39, p. 88, 1953.

Geren, B.B. Formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. Exp. Cell Res. 7, p. 558, 1954.

Gray, E.G. Tissue of the Central Nervous System. In: Electron Microscopic Anatomy. ed. Kurtz, S.M. Academic Press, New York, 1964.

Ham, A.W. and T.S. Leeson. Histology. J.B. Lippincott Co., Phila., p. 491, 1961.

Held, H. Untersuchungen Über den feineren bau des ohrlabyrinthes des Wirbetiere. Abhandl. Math.-phys. Cl. k. sachs Gesellsch: Wessensch. 31, p. 193, 1909. Cited in: Rossi, 1961.

Hess, A. The fine structure and morphologic organization of non-myelinated nerve fibers. Proc. Roy. Soc., B. 144, p. 496, 1959.

Hilding, D. and Jan Wersall. Cholinesterase and its relation to the nerve endings in the inner ear. Acta Oto-Laryng. 55, p. 205, 1961.

Hilding, D., A. Sugiura, and Y. Nakai. Deaf white mink: Electron microscopic study of the inner ear. Annals of Oto.., Rhino., and Laryng. 76, p. 1, 1967.

Holmstedt, B. A modification of the thiocholine method for the determination of cholinesterase. J. Pharmacol. Exp. Ther. 114, p. 167, 1957.

Hursh, J.B. Conduction velocity and diameter of nerve fibers.

Am. J. Physiol. 127, p. 131, 1939.

Iurato, S. Efferent fibers to the sensory cells of Corti's organ. Exp. Cell Res. 27, p. 162, 1962.

Kikuchi, K. and D. Hilding. The development of the organ of Corti in the mouse. Acta Oto-Laryng. 60, p. 207, 1965.

Kimura, R. and J. Wersall. Termination of the olivo-cochlear bundle in relation to the outer hair cells of the organ of Corti in the guinea pig. Acta Oto-Laryng. 55, p.11, 1962.

Koelle, G.B. and J.S. Friedenwald. A histochemical method for localizing cholinesterase activity. Proc. Soc. Exp. Biol. and Med. 70, p. 617, 1949.

Koelliker, A. Handbuch des mikroscopisches anatomie. Engelmann, Leipzig. 1859. Cited in: Rossi, 1961.

Kupfer, C. and G.B. Koelle. Histochemical study of cholinesterase during the formation of motor endplates in the albino rat. J. Exp. Zool. 116, p. 397, 1957.

Larsell, O., McCrady, E., Jr., and Larsell, J.F. The development of the organ of Corti in relation to the inception of hearing. Arch. Otolaryng. 40, p. 233, 1944.

Larsell, O., McCrady, E., Jr., and Zimmermann, A.A. Morphological and functional development of the membranous labyrinth in the opossum. J. Comp. Neurol. 63, p. 95, 1935.

Luse, S.A. Formation of myelin in the central nervous system of mice and rats as studied with the electron microscope. J. Biophys. and Biochem. Cytol. 2, p. 777, 1956.

Lewis, P.R. The distribution of cholinergic neurons demonstrated with the electron microscope. J. Cell. Sci. 1, p. 381, 1966.

Mall, F.P. The branchial clefts of the dog. Studies from Biol. Lab. of Johns Hopkins Univ. 1888. Cited in Streeter, 1907.

Maturana, H.R. The fine anatomy of the optic nerve of Anurans- an electron microscopic study. J. Biophys. and Biochem. Cytol. 7, p. 107, 1960.

Mikaelian, D. and R.J. Ruben. The development of hearing in normal CBA-J mice. Acta Oto-Laryng. 59, p. 451, 1965.

Münzer, F.T. The discovery of the Cell of Schwann in 1839. Quart. Rev. Biol. 14, p. 387, 1939.

Nomura, Y. and H.F. Schuknecht. The efferent fibers in the cochlea. Ann. Otol., Rhinol., and Laryng. 74, p. 289, 1965.

Palade, G.E. The fine structure of mitochondria. Anat. Rec. 114, p. 427, 1952.

Papez, J.W. Superior olivary nucleus: Its fiber connections. Arch. Neur. and Psych. 24, p. 1, 1930.

Pearse, A.G.E. Histochemistry, Theoretical and Applied. Churchill, London. 1960.

Peters, A. and A.R. Muir. The relationship between axons and Schwann cells during development of peripheral nerves in the rat. Quart. J. Exp. Physiol. 44, p. 117, 1959.

Peters, A. Radial component of central myelin sheaths. J. Biophys. and Biochem. Cytol. 11, p. 733, 1961.

Peters, A. Further observations on the structure of myelin sheaths in the central nervous system. J. Cell. Biol. 20, p. 281, 1964.

Peterson, E.R. and M.R. Murray. Myelin sheath formation in cultures of avian spinal ganglia. Am. J. Anatomy. 96, p. 319, 1955.

Portmann, G., M. Portmann, and C. Portmann. La double innervation de l'organe de Corti. Acta Oto-Laryng. 43, p. 226, 1953.

Ramon y Cajal. Histologie du Systeme Nerveux de L'homme et des Vertebres. A. Maloine, Paris. 1909.

Ranvier, M.L. Lecons sur L'histologie du Systeme Nerveux. F. Savy, Paris. 1878.

Rasmussen, G.L. An efferent cochlear bundle. Anat. Rec. 82, p. 441, 1942.

Rasmussen, G.L. Further observations of the efferent cochlear bundle. J. Comp. Neur. 99, p. 61, 1953.

Rasmussen, G.L. Efferent fibers of the cochlear nerve and cochlear nucleus. In: Neural Mechanisms of the Auditory and Vestibular Systems. ed. Rasmussen, G.L. and W.F. Windle. C.C. Thomas Co., Springfield. 1960.

Reichert, C.B. Beitrag zur feineren anatomie de Gehörschnecke des

menschen und der saugethiere. Abhandl. d. Kgl. Akad. d. Wiss., No. 1, p. 1, 1854. Cited in: Streeter, 1918.

Reissner, E. Zur kenntrissde schnecke im Gehor-organ der sougethiere und des menschen. J. Muller's Arch. f. Anat., Physiol., u. Wiss. Med. p. 420, 1854. Cited in: Streeter, 1918.

Retzius, G. Über das Epithel des Sulcus Spiralis Cochleae. Biol. Untersuch, Stockholm, 1884.

Rhodin, J.A.G. An Atlas of Ultrastructure. W.B. Saunders Co., Phila. 1963.

Robertson, J.D. Ultrastructure of adult peripheral myelinated fibers in relationship to myelinogenesis. J. Biophys. and Biochem. Cytol. 1, p. 271, 1955.

Robertson, J.D. New observations on the ultrastructure of the membranes of peripheral nerve fibers. J. Biophys. and Biochem. Cytol. 3, p. 1043, 1957.

Robertson, J.D. The ultrastructure of Schmidt-Lantermann clefts and related shearing defects of the myelin sheaths. J. Biophys. and Biochem. Cytol. 4, p. 39, 1958.

Robertson, J.D. The ultrastructure of cell membranes and their derivations. Biochem. Soc. Sympos. 16, p. 3, 1959.

Rosenbluth, J. and S.L. Palay. Electron microscopic observations on the interface between neurons and capsular cells in dorsal root ganglia of the rat. Anat. Rec. 136, p. 268, 1960.

Rosenbluth, J. and S.L. Palay. The fine structure of nerve cell bodies and their myelin sheaths in the eighth nerve ganglion of the goldfish. J. Biophys. and Biochem. Cytol. 9, p. 853, 1961.

Rosenbluth, J. Fine structure of accustic ganglia in the rat. J. Cell Biol. 12, p. 329, 1962.

Rosenbluth, J. Redundant myelin sheaths and other ultrastructural features of the toad cerebellum. J. Cell Biol. 28, p. 73, 1966.

Ross, L.L., M. Bornstein, and G. Lehrer. Electron microscopic observations on myelin sheath formation in tissue cultures of developing rat cerebellum. Anat. Rec. 136, p. 268, 1960.

Ross, L.L., M. Bornstein, and G. Lehrer. Electron microscopic observations of rat adn mouse cerebellum in tissue culture.

J. Cell Biol. 14, p. 19, 1962.

Ross, L.L. In: Electron Microscopic Anatomy. ed. Kurtz, S.M. Academic Press, New York. 1964.

Rossi, G. Acetylcholinesterase in the course of development of the inner ear of a guinea pig. Acta Oto-Laryng. Suppl. 170. Translation Unit. Library. Division of Res. Services. Nat. Inst. of Health. Bethesda, Md.

Rossi, G. and G. Cortesina. The efferent cochlear and vestibular systems in Lepus cuniculus L. Acta Anat. (Basel). 60, p. 362, 1965.

Ruben, R.J., U. Fuch, and W. Hudson. Properties of the eighth nerve action potential. J. Acoust. Soc. Am. 34, p. 99, 1962.

Schmitt, F.O., R.S. Bear, and G.L. Lark. X-ray diffraction studies on nerve. Radiology. 25, p. 131, 1935.

Schmitt, F.O., R.S. Bear, and K.J. Palmer. X-ray diffraction studies on the nerve myelin sheath. J. Cell. and Comp. Physiol. 18, p. 31, 1941.

Schuknecht, H.F., J.A. Churchill, and R.A. Doran. The localization of acetylcholinesterase in the cochlea. A.M.A. Arch. Otolaryng. 69, p. 549, 1959.

Shambaugh, G.E. The development of the membranous labyrinth. Arch. Otolaryng. 3, p. 233, 1926.

Sjöstrand, F.S. On multiple membrane structures in the retinal rods of the guinea pig eye and in nerve tissue. In: Delft Congress on Electron Microscopy. Nejhoff, The Hague. 1949.

Sjöstrand, F.S. The lamellated structure of the nerve myelin sheath as revealed by high resolution electron microscopy. Experimentia. 9, p. 68, 1953.

Smith, C.A. Innervation pattern of the cochlea. Ann. Otol. 70, p. 504, 1961(a).

Smith, C.A., and F.S. Sjöstrand. Structure of the nerve endings on the external hair cells of the guinea pig cochlea as studied by serial sections. J. Ultrastruct. Res. 5, p. 523, 1961(b).

Smith, C.A. A synaptic structure in the hair cells of the guinea pig cochlea. J. Ultrastruct. Res. 5, p. 184, 1961(c).

Speidel, C.C. In vivo studies of myelinated nerve fibers. Int. Rev. Cytol. 16, p. 173, 1964.

Spoendlin, H.H. and R.R. Gacek. Electron microscopic study of the efferent and afferent innervation of the organ of Corti in cat. Ann. Otol. 72, p. 660, 1963.

Stedmann, T.L. Stedmann's Medical Dictionary. Consulting ed.: I. Asimov et al. Williams and Wilkins, Baltimore. 1966.

Streeter, G.L. On the development of the membranous labyrinth and the acoustic and facial nerves in the human embryo. Am. J. Anat. 6, p. 139, 1907.

Streeter, G.L. The histogenesis and growth of the otic capsule and its contained periotic tissue-spaces in the human embryo. Contr. to Embryol. 7, p. 5, 1918.

Uzman, B. Geren and G. Noguerra. Electron microscopic studies of the formation of nodes of Ranvier in mouse sciatic nerves. J. Biophys. and Biochem. Cytol. 3, p. 589, 1957.

Uzman, B. Geren and G.M. Villegas. A comparison of nodes of Ranvier in sciatic nerves with node-like structures in optic nerves of the mouse. J. Biophys. and Biochem. Cytol. 7, p. 761, 1960

Wada, T. Anatomical and physiological studies on the growth of the inner ear of the albino rat. Amer. Anat. Mem. 10, p. 1 ,1923.

Webster, H. de F. and D.J. Spiro. Phase and electron microscopic studies of experimental demyelination. J. Neuropath. and Exp. Neurol. 19, p. 42, 1960.

Weiss, J.M. Mitochondrial changes induced by potassium and sodium in the duodenal absorptive cell as studied with the electron microscope. J. Exp. Med. 102, p. 783, 1955.

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